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COLLOIDAL GOLD CONTAINING THE RADIOACTIVE ISOTOPE Au^{198} IN THE SELECTIVE INTERNAL RADIATION THERAPY OF DISEASES OF THE LYMPHOID SYSTEM

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THE use of colloidal sols containing radioactive manganese in selective internal radiation therapy of diseases of the lymphoid system has been reported in an earlier communication.[1] In the report it was stated that other isotopes might be of therapeutic interest. More recently the release of radioactive isotopes from the chain-reacting uranium pile prompted us to consider the use of a different isotope since Mn^{52} was not available in therapeutic amounts from this source and since the wide choice of isotopes permitted a more favorable selection to be made. In making an appropriate choice it was required that the half life, radiation, and specific activity be suitable (with absence of long-lived contaminants and toxic effects), that the production cost be low, and that the chemical and biological behavior be familiar. These requirements were best met by the radioactive isotope of gold Au^{198}. In this communication the suitability of this isotope for therapeutic use will be discussed, and methods of preparation of the sol, dosage determination, and administration will be described. Clinical results are reserved for a separate communication now in the course of preparation.

HALF LIFE AND CONTAMINANTS

The half life of Au^{198} is 2.73 days.[2] We find that this rate of decay is ideal in therapy since the patient receives three-quarters of the radiation from a given dose in about five and one-half days. The therapist is then in a position to give additional treatment or not, at his own discretion. This permits rather good control of the radiation which is not possible in the case of longer-lived isotopes.

Practical considerations prevent the use at this time of isotopes of much shorter half life than Au^{198}. As decay rates materially increase, transportation must become increasingly rapid, and the possibility of preparing the colloid in one laboratory for delivery to another qualified center for administration is lost. Furthermore as the period of radiation decreases, biologically effective doses must increase, necessitating more elaborate precautions for the protection of the worker. Although not established there is reason to believe

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that the incidence of radiation sickness will increase as more intense doses of radiation are delivered over shorter periods of time.

Before any new radioactive isotope becomes acceptable for therapeutic use, the amount of long-lived contaminants present should be investigated. In the case of gold it is fortunate that no long-lived isotope of this element is known. Thus one must be concerned only with the presence of foreign elements in the original gold from which the isotope is prepared. Those most likely to be present are silver and copper. Traces of silver may give rise to the 225 day isotope of this element. For this reason a decay study was made on a sample of the gold containing the isotope. The decay was first investigated by following the gamma radiation with a Lauritsen electroscope; later the decay was followed by measuring the activity with a Geiger-Müller counter which is predominantly sensitive to the beta rays. It was found that the decay curve was strictly exponential for well over ten half lives at which time the activity became too weak to follow. This is a "clean" isotope only because gold of suitable purity was activated in the first place.

CHEMICAL AND BIOLOGICAL BEHAVIOR

The chemical behavior of gold is as familiar as that of most elements and the preparation of colloidal suspensoid sols of this element is extremely simple. Such sols are stable and will withstand repeated autoclaving at 120° C. They can be concentrated by boiling, and a reasonable amount of dissolved salts does not appear to affect the stability. This is in definite contrast to our experience with sols of manganese oxides which must be dispersed under sterile conditions since they do not withstand subsequent sterilization.

The biological behavior and toxicology of ionic gold are well known. The literature on colloids is less complete. However, the distribution in the rat of intravenously administered colloidal gold sulfide has been studied.[3] Our own investigation[4] has shown that, in the human being, particles of intravenously administered colloidal metallic gold are phagocytosed by reticulo-endothelial cells, with a higher concentration in the spleen than is obtained with manganese. If we assume that it is desirable to irradiate this organ rather completely in most instances the latter feature is a desirable one. In the present case the toxic effects of ionic gold present no problem. Due to the insolubility of metallic gold blood levels are low following its deposition in the body. An additional safety factor is achieved by limiting the amount of gold to 4 mg. or less per dose. In the course of considerable clinical experience we have never observed any effects which might be attributed to gold toxicity.

RADIATION EMITTED

Au^{198} emits negative electrons with a maximum energy of 0.78 mev and two gamma rays with an energy of 0.28 and 0.44 mev have been found.[2, 5] Although a hard 2.5 mev gamma ray is reported it is not intense enough to make shielding difficult. Two inches of lead give ample protection for the largest amounts involved and considerably thinner shields suffice in many in-

stances. The gamma rays are penetrating enough to permit external tracing of activity in the body of the patient by the use of a directional Geiger-Müller counter. Recent studies would indicate that some features about the disintegration of Au^{198} still require further clarification.[5]

PRODUCTION OF RADIOACTIVE GOLD

The isotope is provided by the Atomic Energy Commission and is prepared by exposing metallic gold to the flux of slow neutrons in the chain-reacting pile at Oak Ridge. The reaction is

$$_{79}Au^{197} + {}_0n^1 \rightarrow {}_{79}Au^{198}.$$

The simultaneous factors of abundant parent isotope, high capture cross section, and high neutron flux place this isotope in the high range of specific activities produced by the pile. It is this which permits such high and therefore therapeutically effective radiation doses to be administered in such small amounts of gold. At the present time the limitation in production is set primarily at quantities which may be safely shipped. Even with this limit the cost is roughly only two to five dollars per dose.

PREPARATION AND ADMINISTRATION

The chemical procedure in the case of radioactive gold is extremely simple. No radiochemical separation is required. It is merely necessary to convert the metallic gold to a solution of gold chloride (probably aurochloric acid) and to reduce this once more to metallic gold in alkaline solution by adding ascorbic acid. The presence of gelatin, although not essential, increases the reliability of the procedure.

Gold foil (16 mg.) containing radioactive gold is transferred to a long-necked Kjeldahl flask provided with a female ground joint. This is supported behind two-inch lead bricks and is viewed in a mirror. A few drops of aqua regia are sufficient to dissolve the metal. The solution is then evaporated to dryness. Since high temperature decomposes the gold salt, the flask is evacuated by inserting a male ground joint connected to a vacuum pump. The flask is heated with a water bath and because of its rounded shape safely withstands evacuation. After drying is complete, 50 c.c. of pyrogen-free water and ½ c.c. of Knox P-20 gelatin* are added. The solution is made alkaline by adding 1 to 2 drops of concentrated KOH. A solution of 25 mg. ascorbic acid in 10 c.c. of water is then added; a deep red colloid forms instantly. In all operations the use of long pipettes and tongs provides added safety for the worker. Individual doses are dispensed from a simple pipette constructed from a 50 c.c. Pyrex graduated centrifuge tube of the conical type. The dispensing tip is attached to the bottom of the tube. Its long S shape permits withdrawal from the long-necked flask while the pipette rests behind the protecting lead shield. The open top is drawn down to a small diameter and is connected through a rubber tube to an external syringe by which the pressure may be controlled

*Furnished by Dr. D. Tourtellotte of the Knox Gelatin Protein Products Co., Camden, N. J.

from outside the shield. The volume of liquid may be observed in the mirror. Doses are dispensed into penicillin bottles which are placed in lead-carrying shields. These provide protection during autoclaving and local transportation.

Intravenous administration is facilitated by the use of a saline infusion. The saline solution is run into the cubital vein in the usual manner and after satisfactory flow is established the sol is poured into the solution by the use of tongs. This technique permits the maintenance of a safe clearance at all times.

THE DOSAGE UNIT

The radioactivity of each dose is determined by measuring the gamma ray intensity at a distance of 1 M., using a Lauritsen electroscope.* Measurements are made on an ordinary laboratory bench which introduces minor uncertainties in the radioactivity of samples of variable size and shape due to scattering and to absorption effects. However, studies have shown that the accuracy is sufficiently good for therapeutic work. Sufficient material must surround the sample to remove all beta rays. This is achieved by placing the sample container in a large glass beaker.

Pending the establishment of accurate millicurie standards of radioactive gold it was decided to adopt an empirical dosage unit. This was the amount of radioactive gold which produces a discharge rate of 0.5 small division per minute on our electroscope. The radium discharge rate determined by the manufacturer for this instrument was 2.14 small divisions per minute per milligram of radium at 1 meter. This figure should suffice to compare our instrument with others from this source. However, the lack of sufficient information concerning the technique of their measurement makes the value unreliable in absolute amount.

Before the absolute decay rate for a given sample of Au^{198} can be established with certainty it will be necessary to investigate the decay characteristics of the isotope further. However, if it can be assumed that the two gamma rays of Au^{198} are emitted in cascade at each disintegration and that the effect on the electroscope of each quantum is proportional to the energy of the quantum, it may be estimated from the response of the instrument to accurately calibrated radium needles of known filtration that one unit is roughly 0.3 millicuries. This estimate is subject to a number of uncertainties. In any event, conservative initial dosage is recommended in order that the response to radiation can be established to the satisfaction of the therapist.

SUMMARY

Colloidal gold containing the radioactive isotope Au^{198} has been used in therapy of diseases of the lymphoid system. The material is obtained at low cost from the chain-reacting pile at Oak Ridge. The isotope has an ideal half life and good radiation characteristics. The chemical procedures are simple, and extremely stable colloids can be prepared. Toxicity effects due to gold are

*Manufactured by the Fred C. Henson Co., Pasadena, Calif.

not encountered. The technique for preparation, dosage measurement, and administration is described. Clinical results are reserved for a later communication.

We wish to thank the Isotopes Branch of the United States Atomic Energy Commission and the cooperative personnel of the Clinton Laboratories who made possible the use of the radioactive material investigated in this research.

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DIRECT INFILTRATION OF RADIOACTIVE ISOTOPES AS A MEANS OF DELIVERING IONIZING RADIATION TO DISCRETE TISSUES

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ELSEWHERE[1, 2, 4] we have described the use of radioactive colloids of Mn^*O_2 and Au^* in an attempt to radiate specifically the lymphoid-macrophage system. The MnO_2 sols were found to be gradually liberated, with evidence of secretion by way of the biliary tract. In the case of the gold sols, however, it was found by use of a directional type of counter[3] that the radioactive material remained in the tissues in which it had been deposited (that is, chiefly the liver and the spleen) for as long as measurements could be made.[5] Because of the chemical characteristics of metallic gold it was felt advisable to determine whether the insolubility of gold colloids in body fluids could be used to advantage in direct infiltration of neoplastic tissue.

When radium needles or radon seeds are implanted in tissue in order to provide localized radiation, it is necessary to filter the alpha and beta emanations in order to avoid too intense radiation immediately about the needle or seed itself. Dependence is then placed on the cross fire of gamma radiation to the affected tissue, but some radiation of the adjacent normal structures cannot be avoided. In utilizing the radiation from colloidal sols directly infiltrated into the tumor tissue, many millions of "point sources" of radiation are involved in emitting beta rays diffusely throughout the injected volume of tissue. Since the mean free path of the beta ray is of the general order of a millimeter, any considerable irradiation of normal surrounding tissue is avoided. In the case of either Au^{198} or Au^{199} isotopes one is not too greatly concerned with the gamma radiation per se since the ionization produced is not of great consequence in comparison with that derived from the beta radiation. However, the gamma radiation is of particular value in enabling the investigator to measure the localization and relative degree of concentration of infiltrated material.

Certain metastatic lesions are so vascular that injection results in effecting an intravenous administration of the colloidal particles, as will be seen in at least one instance below.

Also, some tumor tissue has been found to be so friable or otherwise constituted that it will not contain even relatively small volumes of fluid. In such instances the procedure as outlined in the foregoing becomes untenable. Under such conditions it is felt that resort to other procedures must be taken in order to establish the radioactive material at the desired site. Preliminary investigations are under way to determine whether the propensity for silver to become

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fixed to tissues might be employed to circumvent this problem.[7, 9] Several possible reactions are involved: (1) Reduction of the radioactive silver when injected as the Ag^*NO_3 salt to metallic silver with relatively permanent fixation by the tissue by the presence of reducing materials such as glutathione and cevitamic acid; (2) precipitation of the silver as a chloride by interaction with tissue chlorides; and (3) combination of the silver with the tissue proteins to form relatively insoluble compounds. Silver colloids, especially that of the oxide, also seem promising at the present writing.[9]

Possibilities involved in the use of radioactive silver are legion. Topical application of radioactive silver nitrate is a logical and reasonable approach to irradiation of superficial lesions.

Because Ag^{111} is a pure beta emitter, it is not possible to apply adequately external measurement apparatus for determination of the localization or intensity of radiation from the material deposited deeply in the tissues. This may be circumvented in part by adding small tracer quantities of long-lived $Ag^{108,110}$, but such a procedure must be considered carefully because little is known regarding the rate of removal of silver from tissues under such conditions. In the case of superficial tumors which, following radiation, can be reliably expected to slough off, such a procedure might be justified. The experimental preliminary studies demonstrating the localization of silver in tissues will be reported elsewhere.[7, 9]

METHODS

The preparation of the colloidal sols has been described earlier.[1, 4] Ordinarily the gold sols for intravenous administration were made up to contain 2 Vanderbilt units (V. U.) per milliliter. Such concentrations of radioactivity were usually found to be too low for infiltration work because of the necessity of injecting relatively large volumes of solutions. Therefore the gold colloids were concentrated by boiling so that 5 or more units per milliliter were obtained. The stability of these colloids allows this to be done successfully. It might also be equally practicable to make up the colloid in a smaller volume of solution originally, although difficulties due to "clumping out" of the gold have been encountered occasionally upon attempting to do this.

The "Vanderbilt unit" represents what we estimated at an earlier date to be an approximation of a millicurie and it is an arbitrary unit of dosage. It is now felt to represent nearer ⅔ millicurie. The material is measured at one meter with adequate shielding of beta radiation so that the gamma radiation from Au^{198} only is read on a Lauritsen, radium calibrated electroscope.* Under such conditions a single "Radiation Unit" obtained from Oak Ridge, consisting of 16.0 mg. of gold foil, which has been exposed to a flux of slow neutrons, would have on an average of about 120 V. U. at time of removal from the uranium pile. It has been found that approximately 40 V. U. of Au^{198} administered by vein to an adult of average size may result in a remission in chronic lymphocytic leukemia.[8] However, it must be kept in mind that there is a considerable amount of individual variation in response to such therapy.

In the cases of discrete encapsulated lesions, the colloid was administered in the same manner as would be employed for infiltration of a novocain solution. The long needle, preferably of small caliber was inserted the maximum desired distance into the tissue and the sol released gradually as the needle was withdrawn.

Following infiltration, frequent measurements were made using the directional counter.[3] This instrument consists of two gamma sensitive Geiger-Müller tubes enclosed in about an inch-thick cylindrical lead shield. One of the counters is positioned opposite a removable

*Obtained from the Fred C. Henson Co., Pasadena, Calif.

port whose opening may be varied from one-fourth to three-fourths inch. The other is completely shielded by the lead. The output of these tubes is fed into two counting rate meters which, by an appropriate circuit, are made to subtract. The degree of directionality obtained is quite high. One is able to find the edge of the liver or spleen with ease, and maintenance of injected material in nodes or tumor masses can be readily followed. The whole shielded tube is mounted in a carriage which permits ready translation on all axes as well as rotation.

CLINICAL OBSERVATIONS

E. R., a 28-year-old white man, had been treated for Hodgkin's disease at various times since November, 1945, with x-ray, radioactive colloidal manganese dioxide, nitrogen mustard, and radioactive colloidal gold, respectively. The patient showed good general response to radiation therapy except that there were two persistent enlarged lymph nodes in the right posterior cervical chain. The larger of these measured 3 by 2 by 1.5 cm. and the smaller measured 1 by 1 by 1 cm.; 0.25 ml. (1 V.U.) of colloidal radioactive gold sol was injected into the former and half that quantity into the latter by infiltration. The patient complained of a burning sensation at the site of injection for about one minute, after which the pain ceased. Daily examinations during the ensuing week revealed no swelling, redness, or increased heat in these areas, although there was some tenderness on pressure. One week after the injection there was no decrease in the size of either node. At this time the larger node was infiltrated with 1.5 ml. of the gold sol containing 5 V.U. and the smaller with 0.3 ml. containing 1 V.U. About three hours after injection the patient complained of aching pain in these areas. Redness, swelling and tenderness developed, reaching a maximum in about twelve hours, and gradually subsided. The patient was discharged and was not seen for four months at which time he was readmitted to the hospital. There was an area measuring about 2 by 2 cm. in the right lateral cervical area directly over the site of gold injection which showed a bluish discoloration. There was a slight tenderness in this area and a small pea-sized, nontender, firm node was palpable just inferior to the area of discoloration. No other lymph node enlargement was observed.

H. H., a 57-year-old white man, had had a long standing bronchiectasis and known generalized lymph node enlargement of three years' duration. On Mar. 6, 1946, biopsy of an axillary node revealed lymphosarcoma. Intravenous therapy with radioactive colloidal manganese dioxide was begun and an enlarged lump over the left clavicle diminished somewhat in size as did most of the lymph nodes. There was marked subjective improvement. He was given occasional intravenous injections of colloidal MnO_2 and later of colloidal gold. On Feb. 12, 1947, he was admitted to the hospital for blood transfusion and at this time the supraclavicular mass measured 14 by 12 cm. and was elevated 3.5 cm. above the surface of the surrounding skin (Fig. 1). Seven V.U. of colloidal radioactive gold in 7.0 ml. of solution were infiltrated diffusely into the center of the mass. The patient noted a sensation of constriction in the area for several hours, but no other symptoms were subjectively felt and over a period of twenty-four hours there was no tenderness, increased heat, or redness. A week later there was a definite decrease in the size of the mass centering about the injected area and it was softer. Measurement showed the mass to be 12 by 10

centimeters. Use of the directional counter for measurement of gamma ray activity showed good localization of the radioactivity in the mass. At this time an additional 12 V.U. of sol in 9.0 ml. of solution were infiltrated into the tumor tissue in the upper mid-portion; the patient again complained of a feeling of

Fig.1.—Fibrosarcoma prior to treatment.

Fig. 2.—Fibrosarcoma four weeks later.

tightness and constriction but nothing else. Another 14 V.U. were infiltrated on Feb. 26, 1947, and an additional 7 V.U. on Mar. 19, 1947.

The infiltrated mass had completely disappeared at examination on April 1, 1947 (Fig. 2).

G. S., a 35-year-old white woman (gravida ii, para i, abortus i), was admitted to the gynecologic service May 5, 1947. Five months previously she had received a supravaginal hysterectomy and bilateral salpingo-oöphorectomy for vaginal bleeding. She was found at this admission to have a Group 4 squamous cell carcinoma of the cervical stump (pathologic grade 3). Pelvic examination showed an asymmetrical, nodular cervix which showed gross evidence of carcinomatous slough and growth. The lesion measured 3.5 by 3.5 cm., with marked necrosis of the right posterior lip and central portion. The tissue was very friable and bled easily on manipulation. Infiltration of the parametria was found all the way to the pelvic wall on both sides. On May 8, 1947, 11 V.U. of radioactive colloidal gold sol (3.5 ml.) were injected into the left lateral portion of the cancer mass. The right half was not infiltrated to serve as a control. A lead-shielded 2 ml. syringe with a 22-gauge needle was used for the injection in a manner such as employed for local anesthesia. A vaginal pack was inserted and all towels and operative packs were collected for determination of radioactivity lost by leakage. On May 9 the pack was removed and a reading on the Lauritsen electroscope showed that there was no significant (less than 0.5 V.U.) loss of material.

Observation of the site of injection showed no appreciable change until May 15 when two observers noted a slight area of redness on the left as contrasted with the right. Biopsy was taken of the injected and of the control areas. No difference was noted except some increase in mitoses in the untreated area which was not considered significant. On May 10, 13, and 18 measurements by means of the directional differential counting rate meter[3] showed that the radioactive material was well localized in the pelvis and neither the liver nor spleen showed any radioactivity.

On May 21, 44 V.U. of gold sol (13 ml.) were infiltrated into the entire carcinomatous mass. There was a moderate degree of bleeding during the procedure and some of the radioactive material was seen to ooze from the injected site. It was felt that 13 ml. were too large a volume of solution for this size of growth. Vaginal packs removed the following day, as well as packs saved at the time of injection, showed a loss of 24 V.U. of material. On May 23, localization studies with the directional counter showed very small amounts of activity in the hepatic and splenic areas, but most of the radioactivity was demonstrable in the pelvis. Similar findings were noted on May 25. The patient showed no systemic reaction to the radioactive material and was discharged on May 29, 1947, to be followed in the outpatient clinic. The patient died at home on July 13, 1947.

M. B., a 20-year-old unmarried colored woman (gravida iii, abortus iii), while under treatment for secondary syphilis was found to have squamous cell carcinoma of the vagina. On May 22, 1947, the patient was found to have a friable, fungating mass approximately 4 by 6 cm. in the posterior vaginal wall. On May 28, 60 V.U. of radio gold sol were infiltrated. Operative packs, as well as the vaginal pack removed the following day, showed a loss of 57 V.U. of gold,

leaving a negligible amount in the injected mass. However, on May 31 what material was left in the tumor was found to be well localized by means of the directional counter.

On June 5, 1947, 65 V.U. of radio gold sol (4 ml. of solution) were infiltrated into the carcinomatous mass, but seepage collected in the packs showed a loss of 50 V.U., leaving only 15 units in the tissue. There was no systemic reaction to the radioactive material.

It was apparent that the extreme friability of this tissue precluded the retention of the gold sol. The patient was later treated with interstitial implantation of radium.

E. T., a 62-year-old colored woman (gravida i, para i), was found to have a far advanced (group 4) squamous cell carcinoma of the cervix. The patient previously had received deep x-ray and radium therapy with no improvement. There had been several episodes of vaginal bleeding and the patient was admitted on May 11, 1947. Pelvic examination showed marked stenosis of the vaginal canal, obliteration of all vaginal fornices, and replacement of the cervix by nodular, necrotic cancer mass. On May 15, 16 V.U. of gold sol (3 ml. of solution) were infiltrated, and later measurement of the packs showed less than 1 V.U. lost by seepage. Observations with the directional counter on May 17 and 23 showed the radioactive material to be well localized in the pelvis, none being detectable in the hepatic and splenic areas. There was no systemic manifestation attributable to the radioactive material. Daily temperature spikes existing prior to the infiltration showed no change. The patient was discharged on May 28, 1947, to be followed in outpatient clinic. On the tenth day following discharge the patient reported a small hemorrhage of an hour's duration. No significant bleeding occurred in the six weeks following. It was the consensus that there was no significant change in the condition of the carcinomatous mass due to the radioactive material, possibly because of the low dosage employed. The tissue showed the ability to retain the material and it was felt that such local injection of radioactive inert material might offer an approach to the problem of hemostasis in bleeding of far advanced cases of cancer of the cervix.

C. S., a 29-year-old white man, had been under treatment for chronic lymphocytic leucemia for the past year. The patient originally had shown marked hepatosplenomegaly and extensive lymph node enlargement. There were numerous subcutaneous lesions seen on the forehead. These were violaceous in color and were nontender, varying in size from ½ to 2 cm. in diameter. In addition there were a number of pinkish-tan areas of infiltration in the skin of the trunk and of both arms, varying in size from 0.5 to 2.5 cm. and averaging about 1 cm. in diameter. While under intravenous treatment with the radioactive manganese dioxide colloid, there was a diminution in the size of the liver and spleen and a reduction in the white cell count to desired limits as well as good general subjective improvement. Nevertheless the subcutaneous lesions were unaffected over the period of a year and, if anything, were increasing in size and number. On Jan. 2, 1947, 0.15 ml. of colloidal radio gold (0.5 V.U.) was injected into each of the two nodules on the forehead. These nodules were carefully measured, together with two adjacent nodes left uninjected as controls.

Six days later the patient reported no subjective change since the injection except persistent itching in the injected nodes, but no redness, pain, or increased heat. Examination showed no change in the size of the nodes in the horizontal plane. Palpation, however, revealed a definite hollowing out effect in the center of each injected nodule. The nodes which had measured about 2.0 cm. in diameter were depressed and softened in an area measuring about 1.0 cm. in diameter. There was no redness or tenderness. At this time, 0.25 ml. (1.0 V.U.) was injected into each of three new nodules of about 2 cm. diameter on the forehead. The patient complained of a stinging sensation at site of each injection. The next week the three nodes injected were flat, although the outline of each was clearly distinguishable on the surface of the skin and the dimensions in the horizontal plane remained the same. There was a grayish-black discoloration of the skin which measured about 1.0 cm. in diameter immediately overlying the site of each injection. There was no redness, tenderness, swelling, or increased heat in any of these areas. At this time, 2.0 V.U. in 0.3 ml. of solution of colloidal gold were injected into each of the two most posterior nodules on the top of the head. The next week, on examination, the last two nodules injected were entirely flat. There was no pain or tenderness or local tissue reaction at the injected site.

The following week the patient was readmitted to the hospital for blood transfusion and supportive therapy. On this occasion some preliminary attempts at topical application were carried out. The gauze portion of several Band-Aids was soaked with radio gold in the form of the chloride and placed in position over several of the lesions on the upper back. Each Band-Aid contained about 2.0 V.U. of radioactive material. During the ensuing three days a small area of erythema began to develop in each of the treated areas and the patient was aware of a progressive increase in tenderness. On the third day the Band-Aids were removed. At that time each area of erythema measured approximately 3.0 cm. in width. The inflammatory reaction became progressively more marked and at the end of two weeks there was a crust of dry tissue measuring about 2.0 by 3.0 cm., this being surrounded by a ring of erythema of about 2 cm. width. The lesions at this stage were nontender, dry, and clean. During the following week the crusted portions of the lesions underwent desquamation, leaving underneath an area of smooth, pink, normal-appearing skin in which all gross evidence of leucemoid infiltration had disappeared.

On a number of later occasions subcutaneous nodules were infiltrated with colloidal radio gold, usually 1 V.U. of material being injected in each site. The reaction to the radiation was similar to that seen in the earlier trials.

When the patient reported on May 13, 1947, the most striking change was marked increase in the size of the lympoid masses earlier found in the groin, that on the right measuring 16 by 8 cm. and that on the left 12 by 9 centimeters. Each mass was raised about 2 cm. above the surrounding skin. Both were firm and nontender. Fifteen V.U. of radio colloidal gold in 15 ml. of solution were infiltrated into the mass in the right groin. The transitory burning sensation disappeared after thirty seconds. Eight days later the skin overlying the injected mass showed a dark red discoloration but there was no increased heat or

tenderness in the region. The mass was slightly decreased in size, measuring 14 by 7 cm., and was definitely softer than the uninjected mass on the left. On this date the patient announced that he was leaving town for an indefinite period. When he was readmitted to Vanderbilt Hospital on July 1, 1947, it was found that the "right inguinal mass had diminished greatly in size while that on the left was slightly larger than previously," although the examining house officer did not make any actual measurements. The patient died as a result of hemorrhage into the peritoneum on August 3, 1947.

R. M., a 64-year-old white man with hypernephroma and metastases, first was admitted to Vanderbilt Hospital with the chief complaint of weight loss. The patient had been well until four months previously when he developed postprandial pain relieved by vomiting. There were also anorexia, epigastric fullness, flatulence, abdominal distention, and constipation. There was a weight loss of forty-five pounds in three months. The pain gradually became more severe and the patient weaker. There was an enlarged supraclavicular node as well as enlarged inguinal nodes. Retrograde pyelograms showed filling defects in the calices of the right kidney. A biopsy of the supraclavicular node revealed a hypernephroma metastatic to the node. On Jan. 15, 1947, 45 V.U. of radio colloidal gold diluted in 400 ml. of saline were administered intraperitoneally by slow drip. The patient tolerated the procedure well and complained of no burning or pain at the site of the injection or in the abdomen. During the ensuing five days repeated determinations of the localization of the radioactive material were made with the directional counter. These showed that the material was diffusely spread throughout the cavity and that the majority remained in the peritoneum until radioactive decay was nearly completed. Very little radioactivity could be demonstrated in the liver and none in the spleen, in which sites the gold would be expected to lodge if it were passed via the lymphatics of the diaphragm into the circulation.[6] At no time did the patient complain of any abdominal pain, nor did repeated examinations reveal any tenderness or spasm. The patient was followed in the outpatient clinic; his course was gradually downhill.

C. B., a 52-year-old white woman, had a seven months' history of hypernephroma. The patient had been treated with radioactive colloidal gold by vein on two previous occasions and that material was well localized in the liver, the spleen, and the remainder of the lymphoid-macrophage system, as shown by use of the directional counter.[3] On Feb. 5, 1947, the patient was given 20 V.U. of radioactive gold intraperitoneally in the form of the colloid. Three hours after the injection the patient complained of a severe diffuse abdominal pain. Examination revealed marked tenderness throughout the abdomen. The pain and tenderness gradually disappeared in the next twenty-four hours and the patient was discharged a day later. Repeated surveys with the directional Geiger-Müller counter during the next ten days showed a high degree of activity spread over the entire abdomen.

On a later occasion a metastatic lesion in the right occipitoparietal part of the scalp was directly injected by infiltration with 4 V.U. of colloidal gold. The

lesion was raised, round, and fluctuant and measured 4 cm. in diameter. The patient complained of no pain or burning following the injection, but subsequent radioactivity measurements over the area showed no localization at the site and suggested that the material was promptly removed by the blood stream.

C. S., a 66-year-old white man, was admitted to the hospital on May 14, 1947, with the chief complaint of "swelling of the left eye, trouble seeing and pain." About three months previously he had noted the first swelling on the left side of the nose and of the inner aspect of the left eye. There was also some visual difficulty and diplopia, and frontal headache without dizziness or fainting spells. There was no discharge or bleeding from the left nostril but during the previous month there had been obstruction of this nostril. There was a

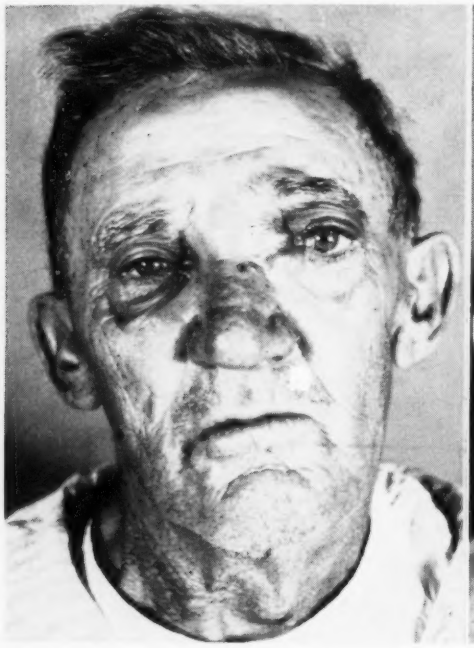

Fig. 3.

Fig. 4.

Fig. 3.—Squamous cell carcinoma prior to local infiltration.
Fig. 4.—Squamous cell carcinoma five weeks later.

weight loss of forty pounds in the previous six months. Three weeks prior to admission the patient reported to a physician who removed "a large amount of tissue" from the nostril and referred him to Vanderbilt Hospital.

A year prior to admission the patient had noted a lump in the throat which "cut off my wind" and produced dysphagia and soreness of throat with difficulty in speech. Shortly before this the patient had noted slight hemoptysis characterized by bright red blood on two occasions. On consulting a physician the patient was told he had a tumor of the throat and sent to Nashville where the tumor was biopsied and forty-three x-ray treatments given on successive days. At the end of this period symptoms entirely disappeared. Three months

before admission the patient noted gradual enlargement of lymph nodes on the left side of the neck as well as those in the inguinal region.

Of chief interest on examination was a pterygium on the left medial side of the eye with slight exophthalmos of the left eye. Along the left side of the nose and along the medial aspect of the left eye was a reddish-blue swelling which extended upon the frontal bone about 1.0 cm. above the eyebrow. The left eyeball was displaced laterally and there was moderate tenderness over the swollen area. The left nostril was filled with gray-colored tumor tissue. The tongue and hard palate were hyperemic and smooth, with mild tenderness. Biopsy showed the lesion to be a squamous cell carcinoma. (See Fig. 3.)

On May 14, the skin over the nose was prepared with iodine and alcohol and novocaine infiltrated into the tumor mass. Nine Vanderbilt units of colloidal radio gold were diffusely infiltrated into that part of the tumor overlying the nose. The tumor mass in the left nostril was also infiltrated with novocaine and 4.0 V.U. of gold were injected. The patient complained of a slight burning sensation in the injected area which disappeared in thirty seconds. On May 21, when the patient reported to the clinic, the external mass over the nose and eye was markedly reduced in size. However, two areas at the medial corner of the eye which were not injected were of the same size. The intranasal mass on the left had almost disappeared. A repeat biopsy was taken from the posterior nares. Radio colloidal gold was injected into the area around the medial corner of the left eye, a total of 11 V.U. in about 3.5 ml. of solution being used. An additional 3.5 V.U. were injected into the intranasal tumor on the left. (See Fig. 4.)

DISCUSSION

Direct infiltration of insoluble radioactive colloidal sols of gold is apparently a suitable manner in which to deliver a given amount of radiation to a discrete mass of tissue under certain circumstances.

X-ray can be delivered only from an external source and, except in superficial lesions, must traverse normal tissues. In tumors originating in deep tissues of the body, a considerable thickness of normal tissues must receive extensive irradiation in order to obtain effective dosage in the tumor. In fact, if the lesion is very resistant to irradiation and deeply seated in the body, effective treatment cannot be delivered by x-ray because of extensive damage produced in the intervening normal structures. In the case of injection treatment with radioisotopes, the treatment is from within the tumor toward the normal tissue. Because of the relatively short path of the beta radiations, considerable effect can be produced in the tumor tissue with relatively little damage to the surrounding normal structures. Injection treatment of deep tissue malignancies may eventually prove to be a very valuable agent in the treatment of cancer. Further experimentation with various malignant tumors and possibly other isotopes is necessary to put this form of treatment on a sound clinical basis.

It was somewhat surprising to us that in the two instances in which gold sols were given intraperitoneally the material remained in the peritoneum, since it had been shown that red cells tagged with isotopes when given by this route find their way rapidly into the systemic circulation by way of the

lymphatic system.[6] It would seem that where generalized diffuse radiation of the peritoneal cavity is desired, as perhaps in abdominal Hodgkin's disease, this might afford a reasonable means of accomplishing it.

In one of the patients with a scalp lesion metastatic to a hypernephroma, injection of the colloidal gold failed to show retention at the site of administration. At autopsy some weeks later it was found that these lesions were traversed by large sinusoids containing blood, and we feel that, in effect, in this case the result was similar to that which would have occurred with intravenous injection.

Most of the subjects treated were far advanced cases in which trial of materials whose correct dosage was unknown was felt justified. In every instance an attempt was made to err on the conservative side and, therefore, we feel that usually the patient was grossly undertreated. However, until more is known concerning the equivalent dosages for various isotopes and until sufficient empirical experience has been obtained to set the correct limits of dosage such a procedure probably will be necessary.

In patient M. B., with carcinoma of the vagina, the character of the tumor mass was such as not to hold injected fluid in a satisfactory manner. Packs removed after instillation and after twenty-four hours showed that practically all of the radioactive colloid had leaked out of this cauliflower-like tissue. Under such conditions it is apparent that the infiltration of gold is highly unsuccessful. We feel that another approach to such problems would be the instillation of $Ag^{111}NO_3$, in which the caustic action of the agent is such as to offer hope of tying it to the desired tissue. Under these conditions we might expect that the silver nitrate might undergo reduction by such compounds as cevitamic acid, glutathione, or any other naturally occurring reducing agent; it might react with the chlorides of the tissue fluids precipitating the AgCl; or it might become bound directly to the tissue proteins. It is well known that in argyria the silver remains in certain sites for extremely long periods of time. Ag^{111} has a pure beta ray spectrum and a half life of seven and one-half days. It is made by transmutation from palladium and therefore has a maximum specific activity eliminating concern of toxic reactions.

The feasibility of use of $AgNO_3$ by direct infiltration has been briefly tested in experimental animals to determine whether it remained at the site of injection.[7, 9] These experiments were carried out using $Ag^{108, 110}$ made by neutron capture in $AgNO_3$. The half life is 225 days and therefore not suitable for human studies. The amounts used in such tracer studies were extremely small (about 57 μg $AgNO_3$) so the conditions were comparable to what might obtain when using the carrier-free Ag^{111} in human therapy.

SUMMARY

1. Under certain conditions it is possible to obtain a high degree of selective radiation of discrete masses of tumor tissue by the direct infiltration of colloidal sols of radioactive gold. In general, the ultramicroscopic particles of gold remain in situ presumably because of their insolubility in body tissue fluids. As a result, a diffuse radiation is possible at any level of tissue ionization desired.

2. Unlike radium implants, it is not necessary to remove the vehicle supplying the source of radiation. Furthermore this material can be infiltrated into tissues which are inaccessible to x-ray and radium therapy, especially should this be feasible in the cases of inoperable tumors found during laparotomy.

3. Cellular structure or vascularity of a tissue may be such as to preclude the retention of solutions injected and the cauterizing properties of $AgNO_3$ are suggested as an alternative, using the isotope Ag^{111}

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METABOLISM OF WOMEN DURING THE REPRODUCTIVE CYCLE

XIV. The Utilization of Pantothenic Acid During Lactation

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MANY studies of the metabolism of pantothenic acid have been made with normal and lactating animals, but little information is available concerning its role in human nutrition. Consequently, the Food and Nutrition Board of the National Research Council was not able to include this vitamin in its list of Recommended Dietary Allowances. Since animal experiments have indicated the importance of this vitamin in reproduction[1-3] and in the growth of young animals,[4, 5] it is likely that it is important similarly in human nutrition. During studies of the utilization of nutriments from food by normal lactating women, pantothenic acid was determined in their food, milk, and urine during the first ten days post partum and during five-day periods at intervals during mature milk production. Other papers have presented the details of the selection of subjects and the organization of the study,[6] the dietary,[7] the method of expressing the milk,[8] and its contents of niacin, pantothenic acid, and biotin,[9] thiamine,[10] riboflavin,[11] ascorbic acid,[12] vitamin A and carotenoids.[13] The proximate composition,[14] lipid[15] and vitamin[16] contents of human placentas have been published as well as the utilization of ascorbic acid,[17] vitamin A and carotenoids,[18] thiamine,[19] riboflavin,[20] and niacin[21] by the nursing mothers. The utilization of biotin is discussed in a following paper.[22] As an adjunct to the study, the amounts of vitamins in the milk and urine of the mothers were determined before and after ten days in which multiple vitamin supplements were added to the diets. The effect of the supplement upon the utilization of vitamin C has been published[23] and its influence upon utilization of other vitamins will be discussed in papers now in preparation.

PROCEDURE

The subjects of the study were multiparas selected during the first trimester of pregnancy on the basis of physical and laboratory examinations and records of having successfully nursed their other children. During all periods of study the women received diets comparable qualitatively but varying in the quantity of all foods, except milk, according to appetite.[7] Different menus were served each day of the five-day study periods and repeated during each period. Aliquot

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portions of each food as eaten were weighed into a composite which was accumulated for each five-day period of study. Both breasts were completely emptied by manual expression at the usual nursing intervals and samples taken for analysis from the complete twenty-four hour collection of milk. The entire excretion of urine during each twenty-four hours also was obtained for analysis.

Seven women were studied throughout the first ten days post partum. Values were obtained for three other women on their seventh days following delivery. Five of the seven women and four others were studied during seventeen five-day periods while they were producing mature milk.

METHOD OF ANALYSIS

The preparation of the milk samples and the general microbiologic procedure of analysis have been described.[9]

Aliquots (400 Gm.) of ground, mixed food composites were homogenized in a Waring Blendor, transferred quantitatively to a 500 ml. volumetric flask, and diluted to volume. Duplicate 5 ml. aliquots (representing 0.6 to 0.7 Gm. of dry food material) were pipetted into one-liter Erlenmeyer flasks and 400 ml. of distilled water added to each. The flasks were autoclaved for fifteen minutes at 15 pounds pressure. When cool, 1 Gm. of mylase P* in 50 ml. of acetate buffer at pH 4.6 was added to each flask, after which they were incubated at 37° for twenty hours. After heating to 100°, the flasks were cooled, their contents adjusted to a pH of 5.3 to 5.7, diluted to 500 ml., and filtered through Whatman No. 42 paper. The filtrate was diluted to a concentration between 1:100 and 1:200 for assay. The values obtained were corrected for the pantothenate in the enzyme.

Urine was filtered, if necessary, brought to a pH of 6.5 to 7.0, and diluted to a concentration 1:100 and 1:200 for assay.

RESULTS

The twenty-four hour milk and urine volumes and the twenty-four hour excretions in urine and secretions in milk during the first ten days post partum are given in Table I. The average values per day for pantothenic acid intake, secretion in milk, and excretion in urine during all five-day periods of study while the mothers were secreting immature and mature milk are given in Table II.

The average daily pantothenic acid intakes during the two five-day periods of study immediately following delivery ranged from 6.0 to 9.5 mg. (Table I). In general, during the first ten days post partum secretion of the vitamin in milk increased in direct proportion to the increase in volume of milk produced. The influence of nutritional status and physiologic state upon the composition of breast milk is indicated by the high concentrations in the milk of V. S., who ingested multivitamin supplements throughout pregnancy in addition to an excellent diet, and by the low values of C. O., who developed a low-grade infec-

*Mylase P is a diastase preparation obtainable from Wallerstein Laboratories, New York, N. Y., which is more active than clarase in liberating pantothenate and contains less pantothenate.[24]

TABLE I. TWENTY-FOUR HOUR PANTOTHENIC ACID EXCRETION IN URINE AND SECRETION IN MILK DURING FIRST TEN DAYS POST PARTUM

SUBJECT	INTERVAL POST PARTUM (DAYS)	VOLUMES		PANTOTHENIC ACID	
		MILK (ML.)	URINE (ML.)	MILK (MG.)	URINE (MG.)
L. F.	1	71	2590	0.03	3.31
	2	200	1275	0.20	3.23
	3	733	2420	1.83	3.80
	4	1122	2075	3.33	4.32
	5	1441	1674	3.89	5.76
	6	1501	1718	3.81	5.51
	7	1596	1340	5.36	4.29
	8	1638	1810	5.45	3.89
	9	1676	1374	6.12	3.03
	10	1872	2220	5.80	4.69
V. G.	1	82	2067	0.02	4.62
	2	177	1689	0.22	4.19
	3	849	1775	1.66	4.98
	4	1413	1323	2.87	3.86
	5	1471	1303	3.06	4.77
	6	1782	1148	4.06	4.73
	7	1630	1230	4.14	4.44
	8	1895	1237	4.30	4.60
	9	1828	1104	4.02	4.15
	10	1770	1075	4.04	4.17
V. K.	1	9	3086	0.00	4.01
	2	90	3651	0.13	4.88
	3	484	2596	1.01	4.70
	4	547	2427	1.30	4.70
	5	560	2331	1.57	5.40
	6	663	2249	1.66	5.12
	7	781	3380	2.05	5.23
	8	775	3840	2.06	5.56
	9	794	3115	1.98	5.13
	10	797	3328	2.19	5.83
V. L.	1	30	1757	0.01	4.28
	2	56	2686	0.03	4.19
	3	353	2759	0.72	4.56
	4	794	1823	1.89	4.92
	5	844	1847	2.55	5.41
	6	955	2710	2.69	5.56
	7	1047	2841	3.65	6.12
	8	1098	2066	3.76	5.40
	9	1118	2151	3.39	4.46
	10	1200	1669	3.72	4.16
J. M.	1	35	2182	0.02	4.42
	2	385	1386	0.53	3.97
	3	870	1519	1.80	4.36
	4	1011	1201	2.40	3.39
	5	1121	1283	2.74	4.45
	6	1125	1172	3.03	4.58
	7	1287	1161	3.70	4.73
	8	1136	1520	3.27	5.58
	9	1258	–	3.26	–
	10	1336	1553	3.82	5.66
C. O.	1	16	1864	0.00	4.20
	2	100	939	0.04	2.98
	3	335	1511	0.22	3.33
	4	595	–	0.61	–
	5	725	–	0.96	–
	6	821	–	1.38	–
	7	798	1470	1.37	4.08

TABLE I. (CONT'D)

SUBJECT	INTERVAL POST PARTUM (DAYS)	VOLUMES		PANTOTHENIC ACID	
		MILK (ML.)	URINE (MG.)	MILK (MG.)	URINE (MG.)
	8	950	1127	1.62	4.92
	9	931	1191	1.58	3.88
	10	660	–	0.77	–
V. S.	1	6	1869	–	5.86
	2	92	1874	0.12	5.11
	3	420	2887	1.10	6.62
	4	600	2063	1.55	5.42
	5	697	2065	1.93	5.88
	6	756	1502	2.36	5.29
	7	818	2524	2.54	6.85
	8	837	2237	3.33	7.59
	9	932	1984	3.26	6.68
	10	924	2287	3.81	6.97
M. S.	7	880	1355	2.99	5.51
G. S.	7	1017	1981	2.29	5.20
F. W.	7	953	1032	1.29	4.17

The first day post partum was variable to the extent of differences in the times at which the women delivered. For subjects delivered after 12 M. the first day began the following morning. The first day for C. O. was 22 hours; for L. F., 23.5 hours. V. G. was delivered eight hours before the beginning of the first day post partum; V. K., seventeen hours; V. L., five hours; J. M., sixteen hours; and V. S., seven hours.

tion near the end of the puerperium. Excretion of pantothenic acid in urine per twenty-four hours was relatively constant for each woman but was not related to urine volume.

The average values per day during all five-day periods (Table II) show the wide variation in milk and urine volumes among the subjects and for the same subject at different times. Although the averages for pantothenate in milk during the two five-day periods immediately post partum portray the large increases in the daily values (Table I) during the first five days, it is interesting that there were only slight differences between the average percentages of intake found in the urine of each woman during the two five-day periods. The greatest difference was an increase from 61 per cent during the first five days to 71 per cent of intake excreted in urine during the second five days by V. S. who had received multivitamin supplements throughout pregnancy. From 70 to 93 per cent of the pantothenic acid intakes of six women during the first five days post partum was accounted for in milk and urine. The range values increased to 94 to 131 per cent for the next five days, with an average increase of 31 per cent. Since the concentration of pantothenate in mature human milk[9] is quite consistent, the values per day in Table II correspond generally with milk volumes. Excretion in urine showed no relationship with urine volume. For all periods of secretion of mature milk from 49 to 103 per cent of the intake was accounted for in milk and urine, with an average of 77 per cent.

DISCUSSION

The average daily urinary excretions of pantothenate for the various five-day periods are comparable to the average daily figures of 4.94 mg. given by

TABLE II. AVERAGE DAILY PANTOTHENIC ACID INTAKE; EXCRETION IN URINE AND SECRETION IN MILK DURING THIRTY-ONE FIVE-DAY PERIODS

SUBJECT	INTERVAL POST PARTUM (DAYS)	VOLUMES		PANTOTHENIC ACID				
				INTAKE	MILK		URINE	
		MILK (ML.)	URINE (ML.)	(MG.)	MG.	% INTAKE	(MG.)	% INTAKE
M. B.	72-76	718	691	6.9	1.17	17	2.20	32
L. F.	1-5	713	2007	8.0	1.86	23	4.08	51
	6-10	1657	1692	7.7	5.31	68	4.28	55
V. G.	1-5	798	1631	6.5	1.56	24	4.48	69
	6-10	1781	1159	6.5	4.11	63	4.42	68
	78-82	848	1281	7.8	2.24	29	4.70	60
	161-165	901	993	8.6	2.16	25	4.72	55
	239-243	681	810	8.0	1.36	17	3.80	48
	302-306	394	1080	7.6	1.05	14	4.74	62
V. K.	1-5	338	2818	6.2	0.80	13	4.74	76
	6-10	762	3182	6.8	1.99	29	5.38	80
	95-99	647	2804	8.6	1.85	21	5.10	59
	144-148	325	1447	8.2	0.98	12	4.50	55
V. L.	1-5	415	2174	8.2	1.04	13	4.67	57
	6-10	1084	2287	9.1	3.44	38	5.14	56
	68-72	789	2849	9.2	2.28	25	3.64	39
	152-156	680	1756	9.4	1.96	21	3.91	42
J. M.	1-5	684	1514	7.9	1.50	19	4.12	52
	6-10	1228	1352	8.9	3.42	38	5.14	58
	75-79	708	2265	9.4	2.13	22	5.12	54
	173-177	268	2003	8.1	0.78	10	4.75	58
C. O.	1-5	354	1438	6.0	0.36	6	–	–
	6-10	832	1263	6.2	1.34	22	–	–
B. S.	85-89	1020	1347	6.8	2.05	30	4.23	62
	204-208	913	1356	7.1	2.09	30	5.18	73
	259-263	676	1219	8.2	1.85	23	5.17	63
G. S.	80-84	899	1078	6.7	2.30	34	3.87	58
V. S.	1-5	363	2152	9.5	0.94	10	5.78	61
	6-10	853	2107	9.4	3.06	33	6.68	71
	70-74	304	1852	6.9	0.88	13	6.17	89
M. S.	58-62	947	939	9.1	2.04	22	4.18	46

Spector and co-workers[25] for normal adult men under "comfortable conditions," but are somewhat higher than the figure 3.5 mg. per day given by Sarett[26] for three normal men on normal diets, or the average values of 3.8, 3.2, and 3.4 mg. found for the normal subjects of Pelczar and Porter,[27] Pearson,[28] and Wright and Wright,[29] respectively. Gardner and associates[30] in a ten-day study, determined the riboflavin, pantothenic acid, and biotin contents of the urine and feces of three healthy young women whose only food other than caramel candy was cow's milk supplemented with a mineral mixture and ascorbic acid. The diet furnished only 6.9 mg. of pantothenic acid daily and the daily urinary excretion of the vitamin averaged 6.0 mg., 87 per cent of the intake. The fecal excretion averaged only 0.41 mg., 6 per cent of intake.

Oldham, Davis, and Roberts,[31] during studies of twelve young women, determined the pantothenic acid content of seventy-two hour collections of food, urine,

and feces from five to nine of the subjects. During consecutive periods of forty-one to fifty-nine days the women received diets which provided average daily intakes of 2.1, 2.1, 3.5, and 4.3 mg. per day, in that order. During these periods the thiamine and riboflavin intakes also were increased from low levels. Three-day composites for each woman were collected during the last week of the study. At the low level of intake, excretion in both urine and feces was quite consistent, averaging 1.6 and 0.8 mg., 79 and 38 per cent of the intake, respectively. With the increased intake, greater variation occurred among individuals and daily excretions in urine and feces rose. However, the average percentage of intake accounted for in urine decreased with greater intake, lowering the total of intake accounted for from an average of 116 per cent during the first two periods to about 94 per cent during the next two study intervals, which may indicate inadequacy of 2.1 mg. of pantothenate per day to supply the metabolic needs of the women.

With the values given in Table II for nursing mothers, the average daily amounts of pantothenate secreted in milk may be subtracted from the corresponding average daily intakes, with the thesis that only the differences are available for use by the mothers' bodies. These net intakes range from 4.94 to 8.56 mg. for the first five days; from 2.39 to 6.34 mg. for the second five days post partum; and from 4.40 to 7.44 mg. during the periods of mature milk production.

From the data for the first ten days post partum it seems evident that the maternal body can store pantothenic acid or that it is retained in the body for more than twenty-four hours during its metabolism. For the first five days post partum the average net intakes of six women exceeded the respective average daily excretions in urine by 0.46 to 2.78 mg.; during the next five days the average daily urinary excretion by four of the women was greater than the average net intakes. Apparently, physiologic levels of pantothenic acid are maintained in both the milk and urine of nursing mothers regardless of current intake if the women's nutritional status with respect to the vitamins is good. Although some workers believe that some pantothenic acid may be available to the body from fecal synthesis, this has not been proved. Since these nursing mothers, receiving a diet providing ample amounts of the vitamin, excreted over 100 per cent of the net intake in urine it seems unlikely that the feces play a significant role in excretion of pantothenic acid intake.

During the periods of mature milk production the average daily secretions in milk and excretions in urine showed no relationship to each other, to urine volumes, or to average daily intakes. Nor was urinary excretion related to milk volume. Although one woman (M. B.) showed an average of only 49 per cent of her intake in urine and milk, from 63 to 103 per cent of intake appeared in urine and milk during the sixteen experimental periods for eight other women. With intakes averaging 6.0 to 9.5 mg. per day, 32 to 89 per cent appeared in the urine, and the variation in urinary excretion occurred in the same individual as well as among the group. It seems doubtful that any great amount of the pantothenic acid was excreted in the feces.

SUMMARY

Pantothenic acid was determined in five-day composites of food and twenty-four hour collections of the milk and urine of seven multiparas during two five-day periods immediately post partum and of nine nursing mothers during seventeen five-day periods at intervals during the production of mature milk.

The average daily intakes of pantothenic acid ranged from 6.0 to 9.5 milligrams. During the first ten days post partum, daily secretion in milk rose with increasing volumes of milk produced. During the five days immediately following delivery, the intakes of six women were 0.46 to 2.78 mg. in excess of secretion in the milk and excretion in urine; during the next five days the milk and urine of four of the women contained averages of more pantothenic acid than their diets furnished. During the periods of mature milk production, 49 to 103 per cent of intake was found in milk and urine. The urine alone contained 32 to 89 per cent of the average daily intakes during the five-day periods.

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METABOLISM OF WOMEN DURING THE REPRODUCTIVE CYCLE

XV. The Utilization of Biotin During Lactation

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SPECIFIC biologic effects of biotin and of the lack of it have been demonstrated in various animals and plants, but there is little indication of its basic function in metabolism. Nevertheless, universal occurrence of this vitamin in living cells, its great biologic potency per unit weight, and the knowledge that it plays an extremely important role in the health and growth of animals and plants suggest an equally important function of biotin in the human being. That biotin may be a component of the enzyme systems concerned with the metabolism of pyruvate or lactate has been indicated by the in vitro tissue experiments of Pilgrim and co-workers[1] and Summerson and associates.[2] That it plays a role as a coenzyme in the transfer of carbon dioxide has also been suggested.[3] In 1941, West and Woglum[4] noted, in rats, that some embryo tissues (skin, muscle, lung, and brain) contained two to thirteen times the concentration of biotin found in corresponding adult tissues. More recently, rat experiments by Kennedy and Palmer[5] have indicated the necessity of biotin for successful gestation, for the birth of viable young, and probably for successful lactation. However, the biotin content of the placenta is singularly low in comparison with the concentration in other organs.[6]

During a comprehensive investigation of the utilization of nutriments from food by normal lactating women, biotin was determined in their food, milk, and urine during the first ten days post partum and during five-day periods at intervals during mature milk production. References are given in a preceding paper[7] to publications containing the details of the selection of subjects and the organization of the study; the dietary; the method of manually expressing the milk; the concentration of niacin, pantothenic acid, biotin, thiamine, riboflavin, ascorbic acid, vitamin A, and carotene in human milk; the proximate composition, lipid and vitamin contents of human placentas; and the utilization of ascorbic acid, vitamin A and carotenoids, thiamine, riboflavin and niacin by the nursing mothers.

PROCEDURE

The subjects of the study were multiparas selected during the first trimester of pregnancy on the basis of physical and laboratory examinations and records

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of having successfully nursed their other children. During all periods of study the women received diets comparable qualitatively but varying in the quantity of all foods, except milk, according to appetite. Different menus were served each day of the five-day study periods and repeated during each period. Aliquot portions of each food, as eaten, were weighed into a composite which was accumulated for each five-day period of study. Both breasts were completely emptied by manual expression at the usual nursing intervals and samples were taken for analysis from the complete twenty-four hour collection of milk. The entire excretion of urine during each twenty-four hours also was obtained for analysis.

Seven women were studied throughout the first ten days post partum. One-day values were obtained for five other women on the seventh, eighth, or ninth days following delivery. Five of the seven women and four others were studied during seventeen five-day periods while they were producing mature milk.

METHODS

The general microbiologic procedure employing *Lactobacillus arabinosus* has been described,[8] including the preparation of food[9] and milk samples for assay.[8]

Numerous control experiments on the food composites showed that maximum values for biotin, representing the sum of the free and bound biotin contents, were obtained by autoclaving the food aliquots in 2 N H_2SO_4 for three hours at 15 pounds pressure. Stronger or different acids or longer periods of heating did not increase the values obtained. Neutralization with NaOH gave values not significantly different from those obtained after neutralization with $Ba(OH)_2$ and subsequent removal of the $BaSO_4$ on filtering. Since the presence of the sulfate ions did not effect the growth of the organism in the assay, NaOH was employed. After neutralization, the autoclaved 5 ml. aliquots were diluted to 500 ml. and filtered through Whatman No. 42 filter paper. The filtrate was diluted further to a concentration suitable for assay, usually 1:200.

The so-called free or loosely bound biotin in the food samples was determined by autoclaving 5 ml. aliquots of the homogenized material with 200 ml. of 0.1 N HCl. When cool, the mixture was brought to pH 5.0 with NaOH, diluted to 250 ml., filtered, and the filtrate diluted to 1:100 or 1:125.

The urine was filtered if necessary and the acetic acid, added as a preservative, neutralized by adjusting the pH to 6.5 to 7.0 before diluting to a concentration of 1:100 to 1:250 for assay.

No attempt was made to differentiate between biotin and its oxygen analogue *dl*-oxybiotin.[10] This vitamer has been shown to have about one-half the activity of *d*-biotin for *L. arabinosus*[11] and perhaps one-tenth of the activity of *d*-biotin for rats.[12] In the only available assays of foods, *dl*-oxybiotin was not found in the materials examined, namely organ and muscle tissues and yeast.[13] Oppel[14] has reported the presence in dog, rabbit, rat, and human urine of a nonavidin-combining biotin, presumably a vitamer.[14] This biotin-like substance was active for his test organism, *Saccharomyces cerevisiae*.[14] It is evidently not oxybiotin, however, since the latter combines with avidin as well as biotin.

RESULTS AND DISCUSSION

The milk and urine volumes per twenty-four hours and the amounts of biotin in the milk and urine of each subject during the first ten days post partum are recorded in Table I.

TABLE I. TWENTY-FOUR HOUR BIOTIN EXCRETION IN URINE AND SECRETION IN MILK DURING FIRST TEN DAYS POST PARTUM

SUBJECT	INTERVAL POST PARTUM (DAYS)	VOLUME		BIOTIN	
		MILK (ML.)	URINE (ML.)	MILK (μG)	URINE (μG)
L. F.	1	71	2590	0.03	18
	2	200	1275	0.05	3
	3	733	2420	0.00	5
	4	1122	2075	0.00	6
	5	1441	1674	0.89	9
	6	1501	1718	2.13	7
	7	1596	1340	0.51	10
	8	1638	1810	0.24	12
	9	1676	1374	0.44	14
	10	1872	2220	1.65	25
V. G.	1	82	2067	0.04	6
	2	177	1689	0.08	9
	3	849	1775	0.36	13
	4	1413	1323	–	13
	5	1471	1303	1.25	43
	6	1782	1148	2.99	33
	7	1630	1230	4.22	32
	8	1895	1237	10.50	37
	9	1828	1104	13.82	31
	10	1770	1075	25.17	59
V. K.	1	9	3086	–	5
	2	90	3651	<0.04	4
	3	484	2596	<0.19	5
	4	547	2427	<0.27	5
	5	560	2331	0.30	14
	6	663	2249	0.52	14
	7	781	3380	0.69	25
	8	775	3840	0.96	20
	9	794	3115	1.31	14
	10	797	3328	3.60	40
V. L.	1	30	1757	–	4
	2	56	2686	–	4
	3	353	2759	–	3
	4	794	1823	–	6
	5	844	1847	–	34
	6	955	2710	0.52	12
	7	1047	2841	0.51	15
	8	1098	2066	0.86	15
	9	1118	2151	1.92	26
	10	1200	1669	12.72	55
J. M.	1	35	2182	<0.02	11
	2	385	1386	<0.19	–
	3	870	1519	<0.87	11
	4	1011	1201	<1.01	16
	5	1121	1283	3.46	68
	6	1125	1172	3.06	30
	7	1287	1161	4.44	29
	8	1136	1520	5.79	38
	9	1258	–	8.25	–
	10	1336	1553	23.65	88

TABLE I. (CONT'D)

SUBJECT	INTERVAL POST PARTUM (DAYS)	VOLUME		BIOTIN	
		MILK (ML.)	URINE (ML.)	MILK (μG)	URINE (μG)
C. O.	1	16	1864	0.00	18
	2	100	939	0.03	4
	3	335	1511	0.10	5
	4	595	–	–	–
	5	725	–	0.44	–
	6	821	–	0.90	–
	7	798	1470	0.72	6
	8	950	1127	0.76	10
	9	931	1191	0.84	12
	10	660	–	1.03	–
V. S.	1	6	1869	–	9
	2	92	1874	–	4
	3	420	2887	–	10
	4	600	2063	0.35	13
	5	697	2065	0.84	39
	6	756	1502	0.98	23
	7	818	2524	1.67	34
	8	837	2237	3.57	40
	9	932	1984	2.63	51
	10	924	2287	23.65	100
M. B.	9	1189	1035	7.49	28
E. L.	8	688	677	5.50	7
M. S.	7	880	1355	0.97	4
G. S.	7	1017	1981	0.64	14
F. W.	7	953	1032	0.70	2

The first day post partum was variable to the extent of differences in the time at which the women delivered. For subjects delivered after 12 M. the first day began the following morning. The first day for C. O. was 22 hours; for L. F., 23.5 hours. V. G. was delivered eight hours before the beginning of the first day of study; V. K., seventeen hours; V. L., five hours; J. M., sixteen hours; and V. S., seven hours.

The values for biotin in milk for the first four or five days following parturition were too low to be reliable. During the second five days both concentration and daily secretion of biotin in milk varied widely among the seven women, ranging from 0.24 μg per day (0.015 μg per 100 ml.) to 25.17 μg per day (2.56 μg per 100 ml.). Some of the values for the fifth and tenth days show abrupt increases, probably owing to the inclusion of liver, which is rich in biotin, in the menus for the fifth day of each period. Although the subjects show small increases in the biotin content of the milk by the fifth day post partum, they are not nearly so marked as on the tenth day.

Unlike niacin[9] and pantothenic acid,[7] biotin excretion in the urine is quite low during the first four days following parturition, increasing several times during the second five days. Increases in urinary excretion on the fifth and tenth days are similar to increased secretions in milk on the days in which liver was included in the diets. Similar changes in the riboflavin content of the milk and urine were noted on those days.[15] It is noteworthy that the milk of L. F. contained very small amounts of biotin during the first ten days post partum,

TABLE II. AVERAGE DAILY BIOTIN INTAKES, EXCRETION IN URINE AND SECRETION IN MILK DURING TWENTY-NINE FIVE-DAY PERIODS

SUBJECT	INTERVAL POST PARTUM (DAYS)	VOLUMES		BIOTIN					
				INTAKE		MILK		URINE	
		MILK (ML.)	URINE (ML.)	FREE (μG)	TOTAL (μG)	(μG)	% INTAKE*	(μG)	% INTAKE*
M. B.	72-76	718	691	42	52	0.9	2	8	15
L. F.	1-5	713	2007	58	88	0.2	0	8	9
	6-10	1657	1692	56	102	1.0	1	14	14
V. G.	1-5	798	1631	63	99	0.4	0	17	17
	6-10	1781	1159	53	67	11.3	17	38	57
	78-82	848	1281	61	88	9.6	11	43	49
	161-165	901	993	56	73	5.1	7	37	50
	239-243	681	810	70	96	4.3	4	40	42
	302-306	394	1080	55	91	4.0	4	40	44
V. K.	1-5	338	2818	47	64	<0.2	0	6	9
	6-10	762	3182	56	66	1.4	2	22	33
	95-99	647	2804	61	78	8.4†	11	52	67
	144-148	325	1447	43	65	1.7	3	24	37
V. L.	1-5	415	2174	65	117	–	–	10	8
	6-10	1084	2287	65	113	3.3	3	25	22
	68-72	789	2849	46	71	5.5	8	29	41
	152-156	680	1756	62	98	6.3	6	39	40
J. M.	1-5	684	1514	64	109	<1.1	1	26†	24
	6-10	1228	1352	60	95	9.0	9	46†	48
	75-79	708	2265	52	83	5.1	6	37	45
	173-177	268	2003	70	117	1.8	2	38	32
C. O.	1-5	354	1438	43	52	0.1	0	–	–
	6-10	832	1263	54	82	0.8	1	–	–
B. S.	85-89	1020	1347	44	68	5.6	8	25	37
	204-208	913	1356	58	80	10.8	14	51	64
	259-263	676	1219	58	75	8.3	11	52	70
M. S.	58-62	947	939	67	83	9.8	12	46	55
G. S.	80-84	899	1078	45	73	6.3	9	29	40
V. S.	1-5	363	2152	61	109	–	–	15	14
	6-10	853	2107	65	93	6.5	7	49	52
	70-74	304	1852	58	82	3.0	4	54	66

*Percentage of intake of total biotin.
†Averages of values for four days.

although in urine she excreted amounts comparable to those of other subjects and by the tenth day was secreting a large volume of milk. That the concentration of biotin in the milk was not lowered by a diluting effect of the high volume secreted is indicated by the values for V. G. who with similar high volume maintained a high concentration of biotin in her milk.

For all five-day periods of study the average daily milk and urine volumes are given in Table II, with the average daily biotin secretions in milk and excretions in urine. For biotin intake both free or loosely bound biotin, as determined after hydrolysis with weak acid for a comparatively short time, and total biotin, the sum of the loosely and firmly bound biotin as determined after hydrolysis with strong acid for long periods of time, are given. It is generally

assumed that the total biotin more nearly represents that available to the animal organism. The biotin in the milk and urine is apparently in the free state, since the values do not increase after hydrolysis with strong acid for long periods of time.

During the periods of mature milk production averages of 2 to 14 per cent of the total biotin intakes were found in the milk secreted during the five-day periods. From 15 to 70 per cent of intake was found in the urine. The diets, which have been described in detail,[16] by analysis provided an average daily intake of 55.8 μg of free biotin and 80.8 μg of total biotin. These values are somewhat higher than those reported for average diets by other investigators. Oppel[17] found daily averages of 30 to 37 μg of total biotin in average diets. Gardner and co-workers[18] reported an average of 33 μg in a moderate-biotin diet which contained no liver or eggs and provided fewer calories and lesser amounts of other vitamins. For diets containing similar amounts of energy and other vitamins, Denko and associates[19] found 37 to 54 μg of biotin, with an average of 44 μg daily. These workers, however, used an enzyme method of extraction[20] which in our hands gave the loosely-bound values for biotin in food composites.

The biotin in milk represented a small percentage of the total biotin in the food during the mature milk periods, ranging from 2 to 14 per cent, in comparison with 10 to 34 per cent for pantothenic acid,[7] 2 to 15 per cent for thiamine,[21] and 3 to 15 per cent for riboflavin.[15] The average figure for biotin excretion in urine during the periods of mature milk secretion was 47 per cent. With the difficulties of complete extraction of biotin from a food composite and the uncertainty as to whether the values obtained accurately represent the amounts available to the subjects who consume the food, it is not surprising that we found little relationship between average intakes within a narrow range, secretion in milk, and excretion in urine by nursing mothers. Together, milk and urine accounted for 54 per cent of the biotin ingested in the food. With daily intakes of 30 to 37μg, Oppel[17] found average excretion values of 29 to 54 μg for normal men, about 85 to 159 per cent of the intakes. Denko and co-workers,[19] working with normal young men, found in urine an average of 32 μg or 73 per cent of the average intake of 44 micrograms. Gardner and associates[18] reported that ten normal young women excreted averages of 29, 30, and 167 μg, or 322, 91, and 98 per cent of intakes of 9, 33, and 171 μg per day, respectively. In an earlier study of young women consuming a mineralized milk diet containing 115 μg of biotin daily, Gardner and co-workers[22] found an average urinary excretion of 50 μg, 43 per cent of the intake.

Fecal biotin may well be a factor in biotin metabolism, since it has been shown that intestinal synthesis is an important source of biotin in many experimental animals. While fecal analyses do not answer the question as to whether the biotin is unabsorbed intake or the product of intestinal synthesis, or to what extent it has been available to the body, Gardner and co-workers[22] found 73 μg, 64 per cent of the intake, in the feces. In another study[19] subjects with intakes of 9, 33, and 171 μg per day excreted 55, 78, and 100 μg in the feces, 611, 236, and 58 per cent, respectively, of the intakes. Oppel[17] found that his subjects on average general diets excreted 86 to 191 μg. of fecal biotin per day.

Denko and associates[19] reported an average fecal biotin excretion of 133 μg daily, 302 per cent of the intake. These investigators noted that 60 to 100 per cent of the vitamins found in the feces were water-extractable.

The results of experimental work with animals and human beings have established the synthesis of biotin by intestinal bacteria. Moreover, the findings that with low intake the urinary excretion of biotin may be much larger than intake and that a large portion of the fecal vitamin is water-extractable indicate availability of fecal biotin to the body. If further work substantiates this, the wide range of response by individuals in a group might be explained by variation in fecal synthesis.

SUMMARY

Biotin was determined in five-day composites of food and twenty-four hour collections of the milk and urine of seven multiparas during two five-day periods immediately post partum and of nine nursing mothers during seventeen five-day periods at intervals during the production of mature milk.

The values for biotin in milk for the first four days following parturition were too low to be reliable. During the second five days both concentration and daily secretion of biotin in the milk varied widely among the seven women, ranging from 0.24 μg per day (0.015 μg per 100 ml.) to 25.17 μg per day (2.56 μg per 100 ml.). During the periods of mature milk production averages of 2 to 14 per cent of the total biotin intakes (0.9 to 10.8 μg per day) were found in the milk secreted during the five-day periods. From 15 to 70 per cent of the intakes (8 to 54 μg per day) was found in the urine.

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A NUTRITION SURVEY OF VIENNESE CIVILIANS UNDER UNITED STATES OCCUPATION, 1945

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THE responsibility for providing in United States occupied Germany and Austria an equitable distribution of available food supplies fell to the Army at the end of World War II. In order to meet this responsibility with the most facts, nutrition surveys were planned well in advance and begun in each area as it was occupied.[1] Teams were organized, each composed of a clinical nutritionist, a nutrition officer (Sanitary Corps) experienced in general matters of food and nutrition and in taking dietary histories, and a laboratory technician. We formed one such team and report here the results of a survey of the nutritional status of the civilian population of Vienna in the six bezirks occupied by United States forces. The survey was conducted from August 27 until September 15, 1945. It was on September 1 that the United States Army officially took over the administration of the area assigned to it. Because of this the dietary survey is divided into two sections; the first including bezirks 7, 8, and 9, which adjoin the inner section of the city, and the second, bezirks 17, 18, and 19, which were in the outlying districts. The first part of the survey was made during the Russian occupation of the entire city and the second after these six bezirks were administered by the American Army. Pyke[2] has reported a similar survey for the British area of Vienna in September, 1945, and the data are quite comparable to those reported here. Following World War I nutrition surveys in Vienna were made under the direction of the American Relief Administration.‖[3, 4]

Consideration will not be given to the previous food intake of the Viennese for two reasons: first, the survey was intended to determine only the food intake at the time the survey was made; and second, reliable figures of food intake during the war were not available. The observations are therefore confined to food intake during the survey itself. It is well known, however, that food was scarce and strictly rationed in Vienna during the war. When the Russian army occupied the city they found that the retreating Germans had left almost no food supplies so that from the end of April until early in June almost no rations at all were issued. During July and August the issue amounted to about 800 calories per day.[2] In general, the Viennese lost weight on rationed food during the war. Thus the Russians, and soon the other members of the four powers.

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found an undernourished population in Vienna, with almost no food available to ration and with grossly inadequate transportation to bring more food. Further, there was an acute shortage of fuel and housing that complicated immensely the public health problems of the city.

MATERIALS AND METHODS

Food Ration Classification.—At the time of the survey the consumers were divided, according to the Russian method of classification, into five food ration categories: "Heavy Workers; Workers; Employees; Children Under Twelve Years of Age; Others." The "heavy workers" included not only those doing heavy manual labor, but also certain professional people such as physicians, opera singers, and orchestra players. The category "employees" was made up chiefly of white collar workers, and that of "others" of all those not in one of the other categories; that is, housewives, disabled persons, and those too old or otherwise unable to work. The estimated number of individuals falling into each category is shown in the first two columns of Table I.

TABLE I

FOOD RATION CLASSIFICATION	APPROXIMATE POPULATION	TOTAL NUMBER STUDIED	MALE	FEMALE
Heavy workers	10,400	115	105	10
Workers	54,500	540	316	224
Employees	29,600	257	93	164
Children under 14	32,400	548	240	308
Others	138,000	865	86	779
Total	264,900	2,325	840	1,485

Composition of the Population of the Zone of United States Occupation.—The United States Vienna Area Command took over the administration of bezirks 7, 8, 9, 17, 18, and 19 on September 1, 1945. Accurate population figures for this area were difficult to obtain, but the closest approximation was about 260,000. The inhabitants of this area varied from the poorest in the city to some of the most wealthy in the suburban districts of Heilengenstadt and Grinsing. Many professional men and women were included as well as workers. The industries located in the area were mostly small.

Selection of the Sample.—With the cooperation of the Public Health Division of the United States Vienna Area Command, the Viennese public health officials and police located central sites for the examination and called in individuals and families to represent as nearly as possible 1 per cent of the total number of each of the four food ration categories. Table I shows that, with the exception of the category "others," roughly 1 per cent was examined in each category. The number of children was large. In all dealings with the civilian officials an effort was made not to mention that the survey was concerned with nutrition. The survey was organized, they were told, in an effort to understand the general level of health of the people of Vienna under the administration of United States forces. It was usually possible to make sure that those asked to come did so and that those ill at home were noted. It was impossible to visit those not appearing because of illness, but the number of such was small. Indeed, a few of those who came should have been in bed at home. Great care

was taken to insure that no greater proportion of ill and undernourished individuals was examined than was present in the population. It was not always possible to be sure of this but we believe that with the method of selection used this error was largely avoided. Bezirks 7, 8, and 9 included a large proportion of the poor of Vienna, those likely to suffer first. However, the findings were not strikingly different in the other three bezirks in which the people were better able to afford food purchased in the black market. It was well recognized that food was available in the black market in Vienna at this time, but the quantity was so small that it could, and apparently did, make but little difference in the nutritional status of those able to afford it.

In order to include a sufficient number of heavy workers and workers, three factories were investigated: an optical works, a clothing factory, and a paper factory.

Procedure of the Examination.—

Physical Examination: Each individual removed his clothing except for underclothes and stockings, was weighed (kilograms) and the height measured (centimeters). The age, sex, height, weight, and food ration category were entered beside the individual's serial number, as were the findings in the clinical examination. Table III shows the items on physical examination which were specifically sought. In addition, inquiries were made concerning menses (in women between 15 and 44 years of age), weakness, and syncope. The following physical abnormalities were sought but so rarely seen that they were not listed in the table: purpura, loss of vibratory sense in the great toes, muscular tenderness, absence of knee jerks, bossing of epiphyses, beading of ribs, and cranial bossing.

Laboratory Examination: About one of every three of the individuals examined was sent to the laboratory technician and his aides who determined blood hemoglobin and serum proteins by the specific gravity method[5] included in the mobile field laboratory.

Dietary Survey:

1. Individual dietary history: Slightly over one-third of the individuals reporting for the examination were questioned as to their food intake during the previous twenty-four hours. Models and drawings of food as well as plates and cups were used as aids. The estimated food consumption was calculated by using the composite of the daily results for each class of consumer. In general, the most reliable histories were given by the housewives who prepared the meals for the entire family. In the two factories studied the food served at work for lunch was investigated and the workers themselves were questioned. Reassurance was given that the examiner had no interest in the source of the food (for example, the black market) but only in its quantity. The frequency with which foods appeared which were obtainable only by illicit means was small, corresponding to the estimated size of black market dealings.

2. Rationing and supply of food in Vienna: The food rationing was administered by civilian food authorities and was well organized. Thus it was possible to obtain the ration for each food without any difficulty.

A good insight into the food supplies and the problems of procurement and distribution were obtained by one of us (H. L. W.) who represented the United States in four power conferences on food at the Russian commissariat. It was thus possible to interpret the clinical findings and to some extent to estimate the future nutritional status of the population.

RESULTS

Clinical Examination.—

Physical Measurements: The heights and weights were averaged for the several groups formed from the different age, sex, and food ration categories. These were compared with standards for the United States.[6, 7] It must be understood clearly that these standards are about 5 kilograms higher than the weight of adult Austrians on a prewar status. Hereafter, the United States standard used will be referred to as the U. S. standard.

Children, taking the 5-, 10-, and 15-year-old groups as examples, averaged not far below the U. S. standard (Table II). This, however, was not true for

TABLE II. AVERAGE HEIGHT AND WEIGHT OF CHILDREN AGES 5, 10, AND 15 YEARS. WEIGHT COMPARED TO U. S. STANDARDS[6]; BOTH HEIGHT AND WEIGHT COMPARED TO SIMILAR MEASUREMENTS, 1920-21[3, 4]

AGE	SEX	HEIGHT (CM.) VIENNA		WEIGHT (KG.) VIENNA		
		1920-1921	1945	1920-1921	1945	UNITED STATES
5	Male	--	103	--	16	17
	Female	--	106	--	16	17
10	Male	--	134	27.6	29	30
	Female	--	135	27.7	26	31
15	Male	151	161	42	47	50
	Female	151	156	44	47	48

TABLE III. SYMPTOMS AND SIGNS SUGGESTIVE OF SPECIFIC DEFICIENCIES EXPRESSED IN PERCENTAGE OF EACH GROUP EXAMINED

	CHILDREN BELOW 14 YEARS		HEAVY WORKERS		WORKERS		EMPLOYEES		OTHERS	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
Poor appearance	7	5	16	0	11	7	17	2	24	7
Thyroid enlargement	38	35	41	40	22	44	33	42	28	36
Folicular hyperkeratosis	7	7	2	0	2	8	0	5	2	4
Cheilosis	0	0.5	0	0	0	0	0	0	0	1
Glossitis	2	0.5	6	10	5	10	12	4	2	6
Abnormal tongue color	1	0.5	8	10	8	7	16	5	6	7
Edentulous*	0	0	38	10	27	19	30	17	31	23
Abnormal color and texture of hands	2	1	18	10	12	10	18	10	20	6
Corneal vascularity	0.5	0	0	0	2	1	1	0.5	1	2
Paresthesia	0	0	12	0	8	4	5	4	8	6

*Not recorded as a sign of deficiency, but only to help interpret tongue changes.

the adults whose weight reduction below the U. S. standard was more marked. Thus, the averages for adults were below the U. S. standard by from 7 to 12 kilograms.

In addition to comparing averages for adults from 21 to 60 years of age, a distribution curve was constructed showing the frequency of occurrence of overweight and underweight. Each individual's weight was compared with the U. S. standard for age, sex, and height, and the number of kilograms below or above the standard was plotted on the frequency distribution curve (Fig. 1). The mean was –9 kilograms, and the mode, –11 kilograms. The reduction below the U. S. standard for weight becomes more striking when it is recognized that almost half (48 per cent) of those examined were more than 10 kilograms below the U. S. standard.

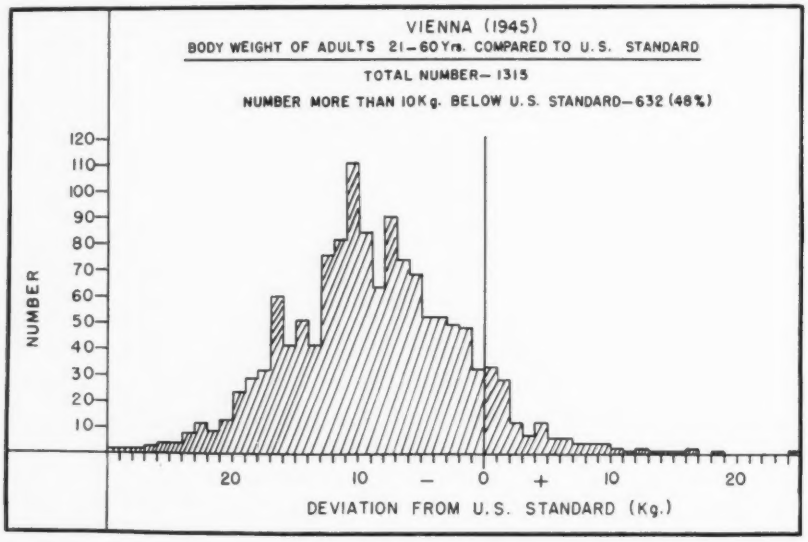

Fig. 1.

Symptoms and General Appearance: Although not specifically tabulated, many individuals spontaneously complained of weakness, fatigue, and even syncope. It was not unusual to see an ecchymosis or a "black eye" and, upon inquiring, it was frequently found that it had followed a fainting spell due to weakness.

The general appearance of the individuals was not that of extreme undernutrition but simply of weight loss. Many appeared normal, a few, plump, but most showed evidence of considerable loss of weight and a few showed extreme weight loss. In the latter, posture was often poor, movements were carried out with a characteristic lethargy, and the facial expression was apathetic. Abnormal pigmentation was not seen.

Symptoms and Signs Suggestive of Specific Deficiency States: Severe vitamin deficiencies were not seen. There was no evidence of beri-beri, peripheral neuritis, or of pellagra, and only one individual with classic scurvy was seen. Certain signs that have been described as suggesting specific deficiencies were

recorded as they were seen. The low incidence of most of these signs is given in Table III. Several signs deserve comment.

Goitre, diffuse, nontoxic, was present in from 21 to 42 per cent of the individuals. It is not known whether this incidence is higher in Vienna at this time than before the war.

Distinct papillary atrophy of the tongue was seen in 156 instances. However, in sixty of these the mouth was edentulous so that the significance of the finding in these individuals is open to question. A beefy red or a magenta colored tongue was observed in 404 individuals of whom 102 were edentulous. The combination of papillary atrophy and either beefy red or magenta colored tongue was seen in thirty-two instances, seventeen being edentulous. Other signs suggesting thiamin, riboflavian, or niacin deficiency were almost absent.

Paresthesia and Acrocyanosis: In studying a small group of starved men we described a condition of the extremities, especially the hands, resembling acrocyanosis, which was frequently associated with paresthesia.[8] The same phenomenon, although with a lower incidence (from 10 to 20 per cent of the adults), was observed in the studies reported here. In the morning, particularly if cold, the hands and feet were cold to the touch and presented a mottled cyanotic appearance. In the warmer part of the day the cyanosis might give way to a mottled reddish flushing, but the hands were usually still cold to the touch. Paresthesia of the hands and feet was associated with acrocyanosis in only forty instances, so that the correlation of the two was not as striking as that found in starved prisoners. The paresthesia was described variously as numbness, tingling, or, often, a burning sensation. These symptoms were most marked in the finger tips and next in the toes; they even extended into the forearms and lower legs.

Edema and Serum Proteins: The serum protein concentration was determined in 859 individuals. The average values for the age and food ration categories are given in Table IV. The individual determinations are plotted as a distribution curve in Fig. 2. It is at once clear that the majority of the serum protein determinations were either normal or slightly reduced and hence the averages were only slightly below normal. Nevertheless, a significant number of individuals were found to have hypoproteinemia. In fact, 28 per cent of the values were 5.5 Gm. per 100 c.c. or below, and over fifty individuals had serum protein concentration between 4.5 and 5.0 Gm. per 100 cubic centimeters. The

TABLE IV. AVERAGE SERUM PROTEIN CONCENTRATION (GM. PER 100 C.C.)

FOOD RATION CLASSIFICATION	AGE	MALE		FEMALE	
		SERUM PROTEIN	NUMBER	SERUM PROTEIN	NUMBER
Heavy workers	19-40	6.8	9	6.3	3
	41-65	6.0	34	6.5	3
Workers	19-40	6.5	26	6.4	64
	41-65	6.4	89	6.5	53
Employees	19-40	6.3	9	6.3	37
	41-65	6.2	32	6.3	41
Others	19-40	6.6	14	6.5	97
	41-65	6.4	35	6.3	203
Children	1-18	6.3	37	6.5	36

hypoproteinemia, although not general, was present in an undoubtedly larger than normal proportion of the population studied.

Dependent edema was observed in 133 individuals. The data are given in Table V. In eighty-eight of these there was an obvious clinical cause for the edema, such as known or evident cardiac or renal disease, or varicose veins. There were forty-five individuals with edema in whom no clinical cause could be found and who were considered to have "hunger edema." The average age of these two groups was the same, but average weight loss was definitely more in the "hunger edema" group. Likewise the serum proteins, although not excessively low, were definitely lower in the latter group. There were also more signs suggestive of other specific deficiencies in the latter group.

Anemia: Blood hemoglobin concentration was determined in 859 individuals. The average values for the age and food ration categories are given in

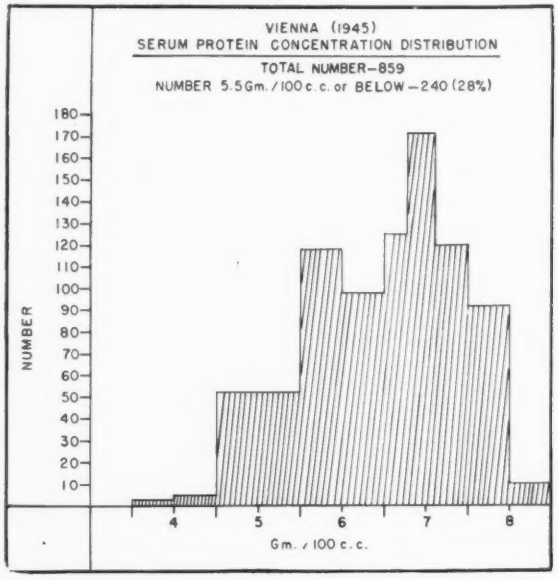

Fig. 2.

TABLE V

	EDEMA	
	WITH OBVIOUS CLINICAL CAUSE	WITHOUT OBVIOUS CLINICAL CAUSE
Number	88	45
Average age	54	54
Males	13	14
Females	75	31
Below U. S. standards (Kg.) (Av.)*	9.2	13.3
Average hemoglobin (Gm./100 ml.)	12.3	13.0
Average serum protein (Gm./100 ml.)	6.4	5.8
Clinical signs† (%)	63	86
Total 133		

*Compared with U. S. standards in War Department Training Manual 8-500.[6]
†Cheilosis, glossitis with atrophy, paresthesia, tongue red or violet in color.

Table VI. The individual determinations are plotted as a distribution curve in Fig. 3. Marked anemia was present in but a very small number. Nevertheless, the average values were definitely below normal. Only in the young male white collar workers was the value above 14 Gm. per 100 cubic centimeters. Thus it appears evident that a mild but definite anemia was present; indeed, a relatively small proportion of the individuals had hemoglobin concentrations of 15.5 Gm. per 100 c.c. or over.

TABLE VI. AVERAGE BLOOD HEMOGLOBIN CONCENTRATION (GM. PER 100 C.C.)

FOOD RATION CLASSIFICATION	AGE	MALE		FEMALE	
		HEMOGLOBIN	NUMBER	HEMOGLOBIN	NUMBER
Heavy workers	19-40	12.9	9	13.4	3
	41-65	13.7	34	13.2	3
Workers	19-40	13.7	26	13.2	64
	41-65	13.4	89	12.7	53
Employees	19-40	14.5	9	13.4	37
	41-65	13.1	32	12.5	41
Others	19-40	13.0	14	12.8	97
	41-65	13.3	35	13.2	203
Children	1-18	12.8	37	13.0	36

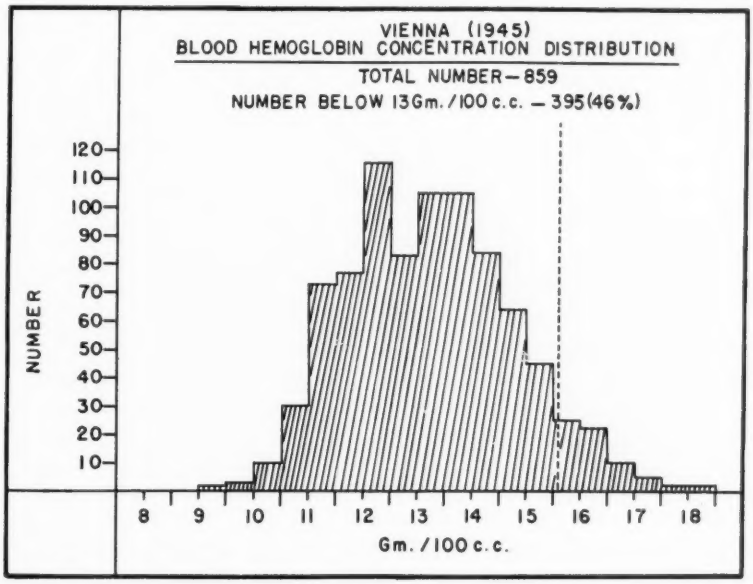

Fig. 3.

Amenorrhea: Absent menses of two months' duration or more, not due to pregnancy, was present in 16 per cent of the women between the ages of 15 and 44. The duration of absence was two to three months in 10 per cent, three to six months in 5 per cent, and more than six months in 1 per cent.

Food Consumption.—

The food consumption of 918 individuals was determined; that of 346 of those from bezirks 7, 8, and 9 is presented in Table VII.

TABLE VII. AVERAGE FOOD CONSUMPTION BY RATION GROUPS, VIENNA, BEZIRKS 7, 8, AND 9, AUGUST 27-31, 1945 (GRAMS PER PERSON PER DAY)

RATION GROUP	NUMBER	BREAD	FLOUR	FATS	POTATOES	GREEN VEG.	OTHER VEG.	LEGUMES	FRUIT	MILK	MARMALADE	SUGAR	BEER
Heavy workers and Workers													
Male	47	267	34	2	183	66	37	84	2		1	1	23
Female	12	332	65	3	104	50	17	58	63			1	
Employees													
Male	34	224	56	2	183	50	38	103	3		2	1	
Female	34	220	66	1	121	22	36	91	2		1	1	9
Children													
0 to 6	24	249	35	1	198	8		77		200			
6 to 12	31	337	32	1	177	12		44		16		8	
Others													
Male	17	221	48	3	215		44	69			1	2	
Female	147	206	39	2	124	24	57	77	6	6		1	6
Total	346												

Although meat, fats, and sugar were authorized for issue, there was no meat available and very little fat or sugar. Most families had none of these items. Likewise, there were no eggs available, with the exception of one individual in an outlying bezirk who reported having had one egg; there was almost no cheese. Consequently, the diet consisted principally of bread, or flour, potatoes, and pulses (dried beans or peas) with some fresh vegetables. Contrary to expectations, the vegetable consumption was not higher in the outlying bezirks (except in the case of the children) with the exception of a few cases where pregnant women or nursing mothers were able to obtain small amounts. The significant fact is that all of the protein of the diets of the people in bezirks 7, 8, and 9 were derived from vegetable sources, and this was also true, with a few exceptions, for the other groups surveyed.

It is also to be noted that ration supplements issued for the worker and heavy worker groups were obviously shared with the entire family, since the bread consumption for children was above the ration allowance, while that for the worker categories was below their ration allowance. This is quite evident when flour is considered a part of the bread ration.

The analyses of the food consumed by ration groups, as found in bezirks 7, 8, and 9, are presented in Table VIII.

It is apparent that the diets as consumed were quite deficient in calories, with the possible exception of children up to six years. Eighty-two per cent of the calories were provided from carbohydrates; whereas it is considered that good nutrition requires not more than 60 per cent of the calories provided from carbohydrates, with the balance from fat and protein. In these diets, fat is notably deficient.

The diets were also deficient in calcium, and in vitamin A riboflavin, and niacin. In most cases the vitamin C content is well below the recommended levels, but, with the exception of the diets for the children in bezirks 7, 8, and 9, the deficiency of vitamin C was probably not serious.

TABLE VIII. ANALYSIS OF FOOD CONSUMED BY RATION GROUPS; BEZIRKS 7, 8, AND 9, AUGUST 27-31, 1945

RATION GROUP	CALORIES	PROTEIN (GM.)	FAT (GM.)	CARBOHYDRATE (GM.)	CALCIUM (MG.)	IRON (MG.)	VIT. A ($\frac{1}{2}$ CAROTENE VALUE)	VIT. B_1 (MG.)	VIT. B_2 (MG.)	NIACIN (MG.)	VIT. C (MG.)
Heavy workers and Workers											
Male	1190	50	6	242	240	15	2360	1.3	0.7	6.4	67
Female	1350	51	9	271	208	14	1850	1.3	0.6	6.1	47
Employees											
Male	1230	54	7	241	257	16	1900	1.0	0.7	6.7	59
Female	1150	50	16	228	180	15	410	1.3	0.6	5.8	38
Children											
0 to 6	1214	52	10	232	383	13	420	1.3	0.8	6.0	26
6 to 12	1260	44	6	255	144	12	400	1.3	0.8	6.0	24
Others											
Male	1100	44	7	219	162	13	430	1.2	0.6	5.4	39
Female	1000	43	6	200	202	12	1270	1.1	0.6	5.1	48

DISCUSSION

From the data presented it is clear that the people of Vienna, as seen in the late summer of 1945, were grossly undernourished. This was evidenced by their appearance, weight reduction (48 per cent were more than 10 kilograms under the U. S. standard (Fig. 1), mild anemia, moderate hypoproteinemia, and "hunger edema" in a small but definite number of persons. It is also clear that the food being eaten at the time of the survey was not sufficient to maintain weight and adequate nutrition. Thus, one can from these facts suspect that frank starvation would have ensued at least by midwinter unless more food was provided. Furthermore, it is probable that with the low vitamin content of the diet some avitaminosis might also be expected. Shipments of food began to arrive, however, and it is reported that this continued throughout the winter and spring of 1946 in sufficient quantities to prevent starvation.

Certain of the observations deserve special comment. The low incidence of the signs associated with specific vitamin deficiencies is a remarkable observation and, in general, agrees with most other reports from Europe.[1, 9] Three factors may help account for this. First, the duration of the very poor diet may not have been long enough to permit the development of signs of deficiency; second, although low in vitamin content, the food did contain some of the B complex, although little C; and third, the metabolic rate was probably significantly reduced. This, as well as the low caloric intake, probably reduced the vitamin requirements.

Loss of weight of adults was by far the most striking finding in the city of Vienna. Nutrition surveys also were made in two smaller cities, Linz (population 250,000) and Enns (population 17,000), by the same group of investigators. In Linz, 22 per cent of the population was more than 10 kilograms in weight under the U. S. standard, in Enns, 18 per cent, as compared with 48 per cent in Vienna. Thus it may be seen that the large city suffered,

as one might expect, much more than the small ones. In a group of German prisoners,[8] 70 per cent were more than 10 kilograms below the U. S. standard. Here frank starvation was present and the incidence of hunger edema was 24 per cent.

It is a little surprising that the average weight of the children was not greatly below the U. S. standard. A similar survey made in 1920 and reported by the American Relief Administration[3, 4] reported values for 10- and 15-year-old children that were lower than those given here, both for weight and height (Table II). It seems probable, therefore, that the nutritional status of the children during World War II was somewhat better than during World War I, and reflects the general increase in height and weight in children in many countries following the first war.

The high incidence of weakness, fatigue, and syncope is not surprising considering the extreme weight loss in many of the adults. The poor posture and lethargy have often been observed in severe weight loss.

It is difficult to interpret the occurrence of papillary atrophy and redness of the tongue since these signs occurred largely in edentulous individuals. Incidence of these signs does not necessarily signify a deficiency in vitamin B complex.

An acrocyanotic appearance of the hands, often with paresthesia, was definite but occurred in a much smaller proportion of the population than was observed in a group of starving German prisoners.[8] This suggests that the phenomenon is related to the degree of undernutrition. Others have described somewhat similar neurological manifestations in undernourished and starved groups and the data have been reviewed recently by Denny-Brown.[10]

The appearance of hunger edema, although in very small incidence, suggests that the nutrition of the general population was at a critical level. The average serum protein concentration was somewhat lower in this group than in those with other forms of edema or in those without edema.

The incidence and duration of amenorrhea in women between the ages of 15 and 44 is probably somewhat increased above that seen in normal individuals in normal times, and is probably related to a number of factors, including undernutrition and stress.

SUMMARY

A survey of the nutritional status of 2,325 Viennese civilians under United States occupation in the summer of 1945 revealed:

1. Marked reduction in weight, especially in adults, below the U. S. standard for height and weight.

2. Commonly observed weakness, easy fatigability, and even syncope; apathetic facial expressions.

3. Surprisingly few signs suggestive of specific vitamin deficiencies.

4. An acrocyanotic appearance of the hands and feet, often associated with paresthesia.

5. Hunger edema in forty-five individuals, although average serum protein concentrations were not far below normal.

6. Average hemoglobin concentrations below normal, although marked anemia was present in but a small number.

Analysis of the average food consumption at the time of the survey showed an intake of from 1,000 to 1,440 calories with about 50 Gm. of protein per day.

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FAILURE OF THE ISOLATED PERFUSED MAMMALIAN HEART TO DESTROY RENIN AND ANGIOTONIN

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IT IS generally assumed that the polypeptide, angiotonin, is the active pressor substance responsible for arteriolar constriction in experimental hypertension and that it is formed by the interaction of the proteolytic enzyme renin on a substrate of plasma globulin. Since the point of pressor activity seems to be directly on the arteriolar muscle, the question of fixation of the pressor material by the effector tissue arises.[1] It is technically difficult to measure fixation by arteriolar muscle, but cardiac muscle is a readily accessible similar material. Consequently, renin and angiotonin were separately perfused through the coronary system of isolated rabbit hearts to determine whether there was any loss of either substance, and saline extracts of perfused and unperfused rabbit hearts were assayed for pressor activity.

Hog renin was prepared from hog kidney powder by the method of Schales.[2] The first or unpurified fraction of renin was used. This renin, which contained 95 mg. per cent total nitrogen, was relatively stable in the refrigerator and its activity was assayed at four cat units per cubic centimeter.* This renin was diluted to 0.5 and 0.05 cat units per cubic centimeter with Ringer-Locke's solution. Rabbits were rendered unconscious by a blow on the head and the hearts were removed and washed in physiologic saline solution at a temperature of 37.6° C. The hearts were immediately attached to a modified Langendorf coronary perfusion apparatus and perfused with Ringer-Locke's solution until they were free from blood, at which time perfusion with renin or angiotonin was begun. Samples of the perfusate as it issued from the venous side were collected at approximately fifteen-minute intervals thereafter until forty-five minutes had elapsed.

The venous perfusate was assayed in cats anesthetized with pentobarbital sodium. Blood pressure changes were read directly from a mercury manometer attached by rubber tubing to a cannulated carotid artery and filled with 4 per cent sodium citrate. The solutions to be assayed were injected into a femoral vein. The higher concentrations of renin were assayed directly as collected from the coronary system, but the more dilute solutions were first converted to angiotonin by the method of Schales, Holden, and Schales.[4] The angiotonin perfusate was also prepared by this method, beef plasma being used as a source of globulin substrate, and was so diluted with Ringer-Locke's solution that 1.0 c.c. gave a 20 mm. rise of blood pressure in the assay cats.

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*One cat unit equals the amount of renin which, if injected intravenously in the anesthetized cat, will produce a blood pressure rise of 30 mm. of mercury.

Different rabbit hearts were used for each dilution of renin and angiotonin. All the perfused hearts were frozen in the refrigerator for varying periods. After refrigeration the hearts were cut into small pieces and ground in a mortar. Each minced heart was then shaken with 20 c.c. of physiologic saline solution for thirty minutes, the mixture centrifuged, and the supernatant fluid assayed for pressor activity in the described manner. Unperfused rabbit hearts were treated and assayed in the same fashion.

Equivalent pressor responses were obtained by the concentrated renin solution before and after perfusion through the isolated rabbit heart (Table I, Experiments 2, 3, 4, and 6). Similarly, following conversion of the dilute renin solution to angiotonin no difference in pressor activity could be demonstrated after perfusion (Table I, Experiments 1 and 5). Equivalent pressor responses were also obtained with angiotonin both before and after perfusion (Table II).

TABLE I. ASSAY OF RENIN IN CORONARY PERFUSATE (INJECTIONS MADE IN ANESTHETIZED CAT)

EXPERIMENT	INJECTION TEST MATERIAL	INJECT. (C.C.)	B. P. RISE (MM. HG)	RENIN LOSS
1	Ringer-Locke's solution	1	0	None
		1	0	
	Unperfused renin, 0.05 cat unit/c.c., converted to angiotonin	2	25	
		1	15	
	Perfused renin, 0.05 cat unit/c.c., converted to angiotonin	2	25	
		2	20	
		2	25	
2	Ringer-Locke's solution	2	0	None
	Unperfused renin, 0.5 cat unit/c.c.	2	30	
	Perfused renin, 0.5 cat unit/c.c.			
	No. 1	2	30	
		2	20	
	No. 2	2	30	
		2	20	
3	Ringer-Locke's solution	2	0	None
		2	0	
	Unperfused renin, 0.5 cat unit/c.c.	2	25-30	
	Perfused renin, 0.5 cat unit/c.c.	2	30	
		2	30	
4	Ringer-Locke's solution	1	0	None
		1	0	
	Unperfused renin, 0.5 cat unit/c.c.	2	30	
		2	30	
	Perfused renin, 0.5 cat unit/c.c.			
	No. 1	2	30	
	No. 2	2	30	
		2	30	
	No. 3	2	25+	
5	Inactivated angiotonin	2	0	None
	Unperfused renin, 0.05 cat unit/c.c., converted to angiotonin	2	18	
		2	12	
	Perfused renin, 0.05 cat unit/c.c., converted to angiotonin	1	10	
		2	20	
		2	15+	
6	Ringer-Locke's solution	2	0	None
		2	0	
	Unperfused renin, 0.5 cat unit/c.c.	2	30	
	Perfused renin, 0.5 cat unit/c.c.	2	25-30	
		2	30	

TABLE II. ASSAY ON ANGIOTONIN IN CORONARY PERFUSATE (INJECTIONS MADE IN ANESTHETIZED CAT)

EXPERIMENT	TEST MATERIAL	INJECT. (C.C.)	B. P. RISE (MM. HG.)	ANGIOTONIN LOSS
7	Ringer-Locke's solution	2	0	None
		2	0	
	Unperfused angiotonin 1:20 in Ringer-Locke's solution	1	20	
		1	20	
	Perfused angiotonin 1:20 in Ringer-Locke's solution			
	No. 1	1	20	
		1	15-20	
	No. 2	1	20	
		1	20	
	No. 3	1	20	
		1	20	
8	Ringer-Locke's solution	1	0	None
	Unperfused angiotonin 1:20 in Ringer-Locke's solution	1	20	
		1	20	
	Perfused angiotonin 1:20 in Ringer's Locke's solution			
	No. 1	1	15-20	
		1	20	
	No. 2	1	20	
		1	20	
	No. 3	1	20	
		1	20	

Saline extracts of both the perfused and the unperfused rabbit hearts were then injected into assay cats and they produced identical pressor responses. Each kind of extract caused an initial drop in blood pressure of 10 to 15 mm. of mercury followed by a rapid rise of 10 to 15 mm. above its original level which lasted less than two minutes. It is assumed that this pressor response is due to the epinephrine-like compound described by von Euler[7] and others.

Renin in a concentration of 0.5 cat unit per cubic centimeter, when perfused through the rabbit heart, caused a decrease in the rate and amplitude of ventricular contraction. On the other hand, a solution whose concentration was 0.05 cat unit per cubic centimeter had no demonstrable effect on the heart. It is believed that the toxic effect of the more concentrated renin was due to the toluene preservative or to other foreign material and not to the renin itself, since others[5] have shown that renin is without effect on the mammalian heart and that toluene in Ringer-Locke's solution is toxic.

Angiotonin, in the concentration used, transiently diminished coronary flow, but a slight augmentation in the rate and amplitude of ventricular contraction was noted. These observations corroborate the work of others.[5, 6]

CONCLUSIONS

1. Renin and angiotonin may be perfused through the coronary system of the rabbit without loss of pressor activity.

2. Saline extracts of normal rabbit hearts and of rabbit hearts which have been perfused with either renin or angiotonin exhibit slight but equal pressor responses.

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POTASSIUM CONTENT OF NORMAL CEREBROSPINAL FLUID

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WITH THE TECHNICAL ASSISTANCE OF M. LUCILLE KEEFER

SINCE the investigations of Mestrezat,[1, 2] reported in 1911 and 1912, the concentration of most of the inorganic elements in normal cerebrospinal fluid has been well known. During the ensuing three decades many workers improved the methods of analysis, adapted them for clinical application, and confirmed the reported concentrations of inorganic elements. The concentrations of the cations sodium, calcium, magnesium, and phosphorus have been well established. The levels of the anions chloride, phosphate, and bicarbonate also can be stated without qualification. The level of the cation potassium, however, has remained more or less in question. For many years the determination of this ion has been of academic interest only, and possibly is even now. For its determination various workers have employed methods derived from chemical reactions which are not ideal for quantitative analysis. Values have been reported after the study of small groups of incompletely described patients. Merritt and Smith[3] concluded that, "the potassium content of the cerebrospinal fluid has not been sufficiently investigated."

In Table I the results obtained by workers who have examined normal spinal fluids have been tabulated. In most cases there was no statement of the estimated accuracy of the method employed, nor has it been possible in every case to ascertain the method used. It may be questioned whether the number of determinations made constituted an adequate series for the formation of statistically significant conclusions.

TABLE I. POTASSIUM CONTENT FOUND BY OTHER INVESTIGATORS

INVESTIGATOR	NUMBER OF CASES	RANGE (MG. PER 100 ML.)	AVERAGE (MG. PER 100 ML.)	METHOD OF ANALYSIS
Leulier, Velluz, and Griffon[4]	20	11 -16	12.3	Modified cobaltinitrite
Pincus and Kramer[5]	3	12.8-16.6	15.1	Cobaltinitrite
Leipold[6]	16	10.5-16.9	13.5	Not stated
Merritt and Smith[3]	15	11 -15	13.2	Not stated
Honeyman and Zwemer[7]	10	12 -14.5	13.3	Modified cobaltinitrite
Eisler[8]	19	13 -13.5	13.2	Cobaltinitrite
Lierle and Sage[9]	4	not stated	12.8	Cobaltinitrite
Duliere[10]	14	not stated	11.17	Cobaltinitrite
Ballif and Gherscovici[11]	not stated	10 -18	not stated	Cobaltinitrite

MATERIAL

The samples of spinal fluid were taken from the lumbar subarachnoid space of patients entirely free from clinical evidence of neurologic disorders. (McCance and Watchorn,[12] in a small series, found that the potassium content of ventricular fluids was slightly less than that

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of fluid from the lumbar sac. Unpublished data of our own indicate a difference in the content at the central and at more distal levels in the same patients.) The ages varied widely from the first to the fifth decade. The number of male and female patients was approximately equal. Many of the specimens were obtained at the time of administration of a spinal anesthetic; that is, the fluid was removed before the agent was instilled. The occasion for the anesthesia was an elective operation such as a hernioplasty. Most of these patients had been fasting twelve hours, but there was no apparent disturbance in fluid or electrolyte balance. The remaining specimens were obtained from patients convalescing on surgical wards. Care was taken to use only those who were in satisfactory general health and without fluid or electrolyte imbalance. No fluid was used from any patient who had a positive blood Kahn reaction.

The fluid was collected in chemically clean test tubes with aseptic precautions. It was placed in the refrigerator until the analysis could be begun. Microscopic examination of the fluid was not made in every case. Ballif and Gherscovici[11] state that the mixture in vitro of cerebrospinal fluid with the erythrocytes of the same individual did not affect the potassium content of the cerebrospinal fluid, even when the mixture was kept from one to four days. All fluids examined in this study were crystal clear.

As a precaution, all fluids were ashed within forty-eight hours of their removal from the patient. It was believed that delay after this treatment of the sample would not be accompanied by any quantitative alteration of potassium content.

It is beyond the scope of this work to criticize the various methods available for the determination of potassium in biological fluids. Peters and Van Slyke, in addition to other authorities, have indicated that the determination of potassium by converting it to potassium chloroplatinate and subsequently iodoplatinate is a method based on sound principles of quantitative analysis. The Lindo-Gladding procedure[13] was adapted by Shohl and Bennett[14] and later altered by Consolazio and Talbott.[15] We have employed their methods with few alterations. The changes made, however, seemed of such importance that we have detailed our steps. We have borrowed freely from Shohl and Bennett and from Consolazio and Talbott for the general outline of the analysis.

METHOD

The principle of the method is the precipitation of potassium chloroplatinate in the ash of biologic fluids and its determination by titration. A quantitative determination is made possible by the conversion of potassium chloroplatinate to potassium iodoplatinate on the addition of potassium iodide. The wine color of potassium iodoplatinate varies in intensity depending upon the amount of potassium chloroplatinate reacting with potassium iodide. The rate of development of the color is proportional to the concentration of the potassium iodide. Heat also speeds the development of color. In the presence of acid color development is hastened, but an excess of acid liberates free iodine which will destroy the proportional relation of color development. Sunshine will also liberate iodine.

In the final titration iodoplatinate is reduced in neutral solution with sodium thiosulfate. This reduced salt is lemon colored and serves as a self-indicator. At the end point one drop of the thiosulfate changes the solution's color from reddish- to greenish-yellow.

Reagents and Special Equipment.—

50 per cent sulfuric acid.

50 per cent sodium sulfate solution.

20 per cent chloroplatinic acid in N HCl. Made by dissolving 10 Gm. platinum chloride, $H_2PtCl_6 \cdot 6H_2O$ in N hydrochloric acid and diluting to 50 ml. with N HCl.

Absolute ethyl alcohol saturated with potassium chloroplatinate. The potassium chloroplatinate was prepared by mixing 0.286 Gm. of potassium chloride, 0.5 Gm. of platinum chloride, $H_2PtCl_6 \cdot 6H_2O$, and 1 ml. distilled water. These were allowed to stand a short time; the supernatant was then drained off by suction, and the precipitate washed twice with 5 ml. of absolute ethyl alcohol. The precipitate was dried by evaporation and kept in a desiccator. To 500 ml.

absolute alcohol was added the amount of potassium chloroplatinate held on a toothpick end. This was shaken and allowed to stand overnight. Without disturbing the precipitate, the solution was filtered immediately before each use. The unused solution was returned to the bottle containing the excess of potassium chloroplatinate.

2 N (approximately) potassium iodide. This was made each time immediately before use and placed in a brown bottle.

0.1 N sodium thiosulfate. The normality required frequent checking.

0.1 N potassium iodate. This was used to standardize the sodium thiosulfate solution.

10 ml. capacity cone-pointed quartz tubes.

Glass stirring rods, 1 mm. diameter and 200 mm. long.

Muffle furnace.

Microburette with fine capillary tips for delivery.

Capillary suction tips for removal of supernatant without disturbing the precipitate.

PROCEDURE

Each determination was completed in one cone-pointed quartz tube. Two blanks were prepared, each containing one drop 50 per cent sulfuric acid and one drop 50 per cent sodium sulfate. Wherever possible, samples were analyzed in duplicate or triplicate. One milliliter of unknown solution, one drop 50 per cent sulfuric acid, and one drop 50 per cent sodium sulfate were placed in the quartz tube. The tubes were kept in the constant temperature oven at 100 to 105° C. until the solution had evaporated; this process usually required four hours. The tubes were transferred to the sand bath with temperature at 100° C. Placing the tubes in the sand at an angle seemed to result in more even distribution of the heat. The sand bath temperature was slowly raised to 260° C. and maintained for not less than twelve hours. The tubes were transferred to a cold muffle furnace and the temperature slowly raised to 475 to 500° C. This temperature was maintained for four hours and it thoroughly ashed the tube contents, producing a fine white powder. During the process of ashing, the residue tended to creep up the sides of the quartz tubes. Slowing the ashing step controlled this creeping tendency and eliminated the error which would have resulted. The tubes were left in the furnace until cool. Results were better if the determination was continued from this stage without delay.

To each tube was added 0.2 ml. of 20 per cent chloroplatinic acid which dissolved all the ash. Five milliliters of filtered absolute alcohol saturated with potassium chloroplatinate were added; the solution was thoroughly mixed and allowed to stand for thirty minutes. The tubes were centrifuged for five minutes at 2,000 revolutions per minute. The supernatant liquid was removed through a capillary tube by suction. The procedure was carried out with gentle suction because a minute portion of the precipitate floats and does not settle during centrifuging. By keeping the capillary tip well submerged and moving it toward the precipitate in the quartz tube base as siphoning proceeds, all the supernatant fluid can be removed without loss of precipitate.

Five milliliters more of the filtered absolute alcohol were added to each tube and mixed well using the capillary stirring rod which accompanied the tube throughout the determination. All yellow color was washed from the precipitate. Each stirring rod in each quartz tube was rinsed with approximately 0.5 ml. of the alcohol. The tubes were again centrifuged and the supernatant removed by cautious suction. If any yellow color remained in the precipitate, the entire washing process was repeated. Only by washing out all the yellow chloroplatinic acid could an accurate final yield of potassium be obtained.

The tubes were placed in a water bath at 70° C. and slowly brought to boil; boiling was continued until all traces of alcohol were evaporated. After each stirring rod had been returned to its proper tube, the precipitate in each was dissolved in 5 ml. distilled water. To each tube was then added 1 ml. 2 N potassium iodide. The tubes were held in the hot bath a few minutes for full color development and then were placed in an ice bath and kept cold until titration was completed. It was found that the reduced tem-

perature limited the oxidation of the potassium iodoplatinate by atmospheric oxygen, rendering the potassium yield more accurate.

In the absence of direct sunlight, each tube was titrated to a lemon yellow with freshly made 0.01 N sodium thiosulfate. Just before use this was made from the 0.10 N standard sodium thiosulfate reagent. It was found that for accurate results it was necessary to complete the titration within thirty minutes after the addition of the potassium iodide solution.

It is of importance to know the accuracy obtainable by this analysis. Shohl and Bennett,[14] employing the chemical reactions outlined, found that 0.1 mg. could be determined ± 4 per cent, and 0.4 mg., ± 2 per cent. Consolazio and Talbott,[15] modifying Shohl and Bennett's work so that the determinations were carried out in one container (a cone-pointed quartz tube) without transfer, believed their average error to be less than 2 per cent. By statistical analysis of the values obtained from determinations on replicate samples it was shown that in the method employed in this investigation the standard deviation was ± 0.27 mg. per cent. Expressed in per cent the analytical error of the method was 2.75.

RESULTS

The results of the study are summarized in Table II. In analysis of crystal clear samples from sixty-four patients who were without fluid or electrolyte imbalance, detectable signs of organic central nervous system disease, or syphilis, the values were found to range from 8.5 to 11.5 mg. per cent. It is seen in Table I that other workers have found ranges differing somewhat from this. There are two differences which preclude comparison of their results and the results we obtained. The number of analyses completed are not of the same order. In most studies the cobaltinitrite method was employed and it is now accepted that in potassium sodium cobaltinitrite the potassium content may vary.

TABLE II. POTASSIUM CONTENT IN SAMPLES OF SPINAL FLUID FROM SIXTY-FOUR NORMAL HUMAN BEINGS (MG. PER 100 ML.)

8.5	9.3	9.8	10.3
8.7	9.3	9.8	10.3
8.8	9.3	9.8	10.3
8.9	9.4	9.9	10.4
8.9	9.4	9.9	10.4
9.0	9.4	10.0	10.5
9.0	9.5	10.0	10.5
9.0	9.5	10.0	10.5
9.1	9.6	10.0	10.6
9.1	9.6	10.1	10.6
9.1	9.6	10.1	10.7
9.1	9.6	10.1	10.7
9.1	9.6	10.1	10.7
9.2	9.7	10.2	10.9
9.2	9.7	10.2	10.9
9.3	9.8	10.2	11.5

Analysis of the normal values gave a mean of 9.8 mg. per cent. The standard deviation of this value was 1.1 mg. per cent; or expressing the standard deviation of the mean in per cent it was 11.2.

SUMMARY

Brief reference to earlier investigations of the concentration of inorganic constituents of normal spinal fluid has been made. Samples of spinal fluid obtained by lumbar puncture from sixty-four normal people were examined for

potassium content. The steps of the analytical method were detailed; these included the formation of potassium chloroplatinate from the potassium contained in the unknown, the conversion of this salt to potassium iodoplatinate, and its final titration with sodium thiosulfate.

The results obtained from the sixty-four normal subjects were tabulated. The range of potassium was found to be 8.5 to 11.5 per cent. A mean of 9.8 mg. per cent was calculated.

Without the assistance of Dr. Henry Ryder it would have been impossible to complete the statistical analysis of results obtained in the preliminary evaluation of our method and its application to normal spinal fluid. Dr. Charles D. Stevens and Dr. Samuel Rappaport rendered invaluable assistance with many problems which arose as the method of chemical analysis was being perfected.

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EFFECTS OF TETRAETHYL AMMONIUM CHLORIDE ON A MIXED TYPE OF HYPERSENSITIVE CAROTID SINUS SYNDROME

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IT IS now well known that hypersensitive carotid sinus reflexes can cause syncope or convulsive seizures in human beings. Weiss and Baker[1] described three types of cardiovascular response to stimulation of a hypersensitive carotid sinus, all of which may cause loss of consciousness. The first of these occurs without changes in arterial blood pressure or pulse rate. It is accompanied by blanching of the face, which is believed to reflect cerebral vasoconstriction sufficient to cause temporary cerebral hypoxia. The second type, occurring particularly in persons with arteriosclerosis, is accompanied by syncope which is preceded by a marked fall in blood pressure with little or no change in pulse rate. The type of cerebral ischemia thus induced is due to arterial hypotension. These two types of response are thought to result from increased activity of the sympathetic nervous system. The third type, the most common, results from reflex increase of vagal tone. During this form of reaction there is marked slowing of the pulse rate without primary change in arterial pressure. The bradycardia and the accompanying cerebral manifestations can be prevented by adequate dosage of atropine sulfate.

Recently, a patient who exhibited signs and symptoms of increased responsiveness to stimulation of the carotid sinus was referred to the Research Division of Cleveland Clinic Foundation. Because of the present interest in the effects of tetraethyl ammonium ion upon functions of the vegetative nervous system, the effect of the drug was investigated in this syndrome.

CASE REPORT

A 52-year-old white man had noted over a period of months brief episodes of giddiness which followed stooping, sudden turning of the head, and shaving his neck. On one occasion he lost consciousness and had a short convulsive seizure. Examination showed grade 1 retinal arteriolar sclerosis. Arterial blood pressure was 130/80 at the first visit but thereafter averaged 110/70. Pressure applied to the left carotid sinus while the patient was sitting caused bradycardia and reproduced the giddiness. Pressure on the right sinus caused marked slowing of the heart and a brief, generalized, clonic seizure which ended when pressure was removed. Examination showed the blood and urine to be normal. The standard limb leads of the electrocardiogram were normal.

Effects of Carotid Sinus Stimulation on Pulse Rate and Blood Pressure.—Striking bradycardia resulted from pressure applied to either the right or left carotid sinus or to both. In one instance, asystole which was prolonged for 5.4 seconds was recorded in standard limb Lead II of the electrocardiogram (Fig. 1). The blood pressure during stimulation fell from control levels of 110/70 to range from 90/60 to 60/50. The degree of hypotension was dependent on the intensity and duration of pressure and on whether one or both sinuses were stimulated. It was thus regularly possible to reproduce the presenting symptom of giddiness.

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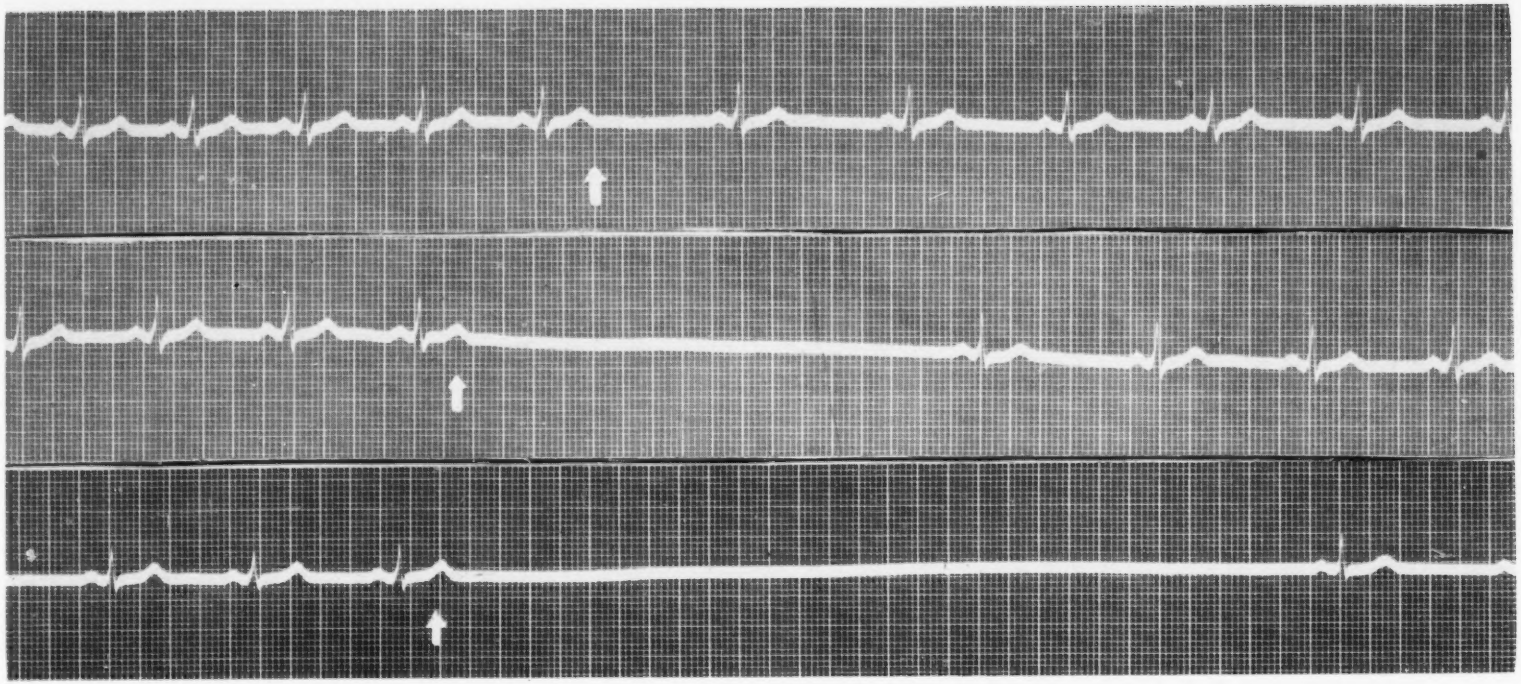
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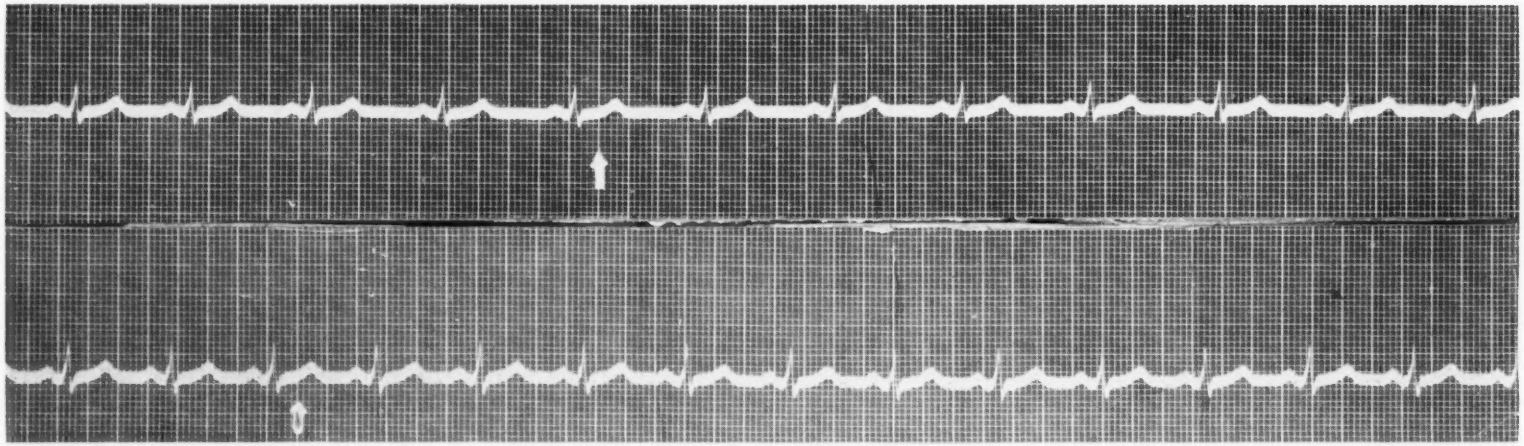

Fig. 1.—Effects of stimulation of carotid sinuses as observed on Lead II of the electrocardiogram. Arrow indicates time of onset of stimulation. 1, stimulation of the left carotid sinus; 2, stimulation of the right carotid sinus; 3, simultaneous stimulation of the carotid sinuses; 4, simultaneous stimulation of the carotid sinuses after intravenous injection of 1.3 mg. atropine sulfate; 5, simultaneous stimulation of the carotid sinuses after intravenous injection of 400 mg. tetraethyl ammonium chloride.

Effects of Atropine Sulfate.—After the intravenous injection of 1.3 mg. of atropine sulfate even severe stimulation of the sinuses did not slow the heart rate appreciably (Fig. 1). Although the blood pressure fell from 102/72 to 84/52, no subjective symptoms appeared (Table I). The persistence of a hypotensive response after atropine had prevented bradycardia indicates that the type of hypersensitivity was mixed, that is, of both second and third types of reaction.

TABLE I. EFFECTS OF STIMULATION OF CAROTID SINUSES BEFORE AND AFTER INTRAVENOUS INJECTION OF ATROPINE SULFATE AND OF TETRAETHYL AMMONIUM CHLORIDE

	CONTROL		AFTER INTRAVENOUS INJECTION OF 1.3 MG. ATROPINE SULFATE		AFTER INTRAVENOUS INJECTION OF 400 MG. TETRAETHYL AMMONIUM CHLORIDE	
	BLOOD PRESSURE (MM. HG.)	PULSE (PER MIN.)	BLOOD PRESSURE (MM. HG.)	PULSE (PER MIN.)	BLOOD PRESSURE (MM. HG.)	PULSE (PER MIN.)
Initial	104/70	83	102/70	84	90/60	91
During stimulation of left carotid sinus	98/66	55	88/68	82	90/60	91
During stimulation of right carotid sinus	68/48	(3.2 sec. asystole)	84/54	80	88/50	88
During simultaneous stimulation of both carotid sinuses	60/50	(5.4 sec. asystole)	84/52	70	88/50	85

Effects of Tetraethyl Ammonium Chloride.—Intravenous injection of 400 mg. of tetraethyl ammonium chloride reduced the arterial pressure on an average from 110/70 to 80/50-90/60 on three occasions. There was slight increase in pulse rate (82 to 90) and the only subjective symptoms were slight and transient numbness and tingling. Two to three minutes after administration of the drug, pressure over the carotid bifurcations caused no change in pulse rate, elicited no symptoms, and the blood pressure fell only 5 to 10 mm. systolic and diastolic. The refractory phase persisted for thirty to forty minutes, at the end of which time stimulation induced the phenomena seen in control observations.

Tetraethyl ammonium chloride (400 mg.) was given by intramuscular injection on alternate days for fourteen days. During this time, the symptoms did not recur. However, it was not unusual for several weeks to pass without symptoms when no treatment was being given.

COMMENT

The mechanism by which tetraethyl ammonium ion blocks the passage of impulse over the autonomic nervous system has been studied by Acheson and Moe.[2, 3] They demonstrated that the drug paralyzes sympathetic and parasympathetic ganglia. Clinical evidences of parasympathetic inhibition were furnished by Lyons and co-workers[4] who demonstrated gastrointestinal hypomotility and loss of tone of the urinary bladder. Birchall, Taylor, Lowenstein, and Page[5] attributed the mydriasis and dry mouth which followed intravenous injection of the drug to paralysis of the parasympathetic ganglia of cranial nerves.

In the light of this evidence it is not surprising that tetraethyl ammonium inhibits the increased vagal responsiveness of hypersensitivity of the carotid sinuses. In contrast to atropine, which acts at nerve endings, tetraethyl ammonium blocks transmission at the ganglia. Nevertheless, the ultimate effects of the two drugs on the parasympathetic component of the hypersensitive carotid sinus reflex are similar. This similarity of action does not extend to the sym-

pathetic aspect of the carotid sinus reflex, namely the hypotension. Atropine does not prevent the fall in blood pressure induced by sinus stimulation. Tetraethyl ammonium, by virtue of its blocking both parasympathetic and sympathetic ganglia, prevents both the vagal component of bradycardia and the arterial hypotension.

These observations furnish further clinical evidence of the activity of tetraethyl ammonium on the autonomic nervous system. However, the effects in hypersensitivity of the carotid sinus are transient. Consequently, the drug is not recommended as a substitute for atropine in the management of hypersensitivity of the carotid sinus, characterized by bradycardia.

SUMMARY

A 52-year-old man who had hypersensitive carotid sinus syndrome was observed. Stimulation of the sinuses by pressure caused bradycardia, reduction of blood pressure, giddiness and syncope. The bradycardia was presumably due to increased vagal tone and the fall of arterial pressure to vasodilatation of sympathetic origin. Atropine sulfate, by its ability to inhibit parasympathetic activity, blocked the vagal but had no effect upon the sympathetic component. Tetraethyl ammonium chloride, which paralyzes both parasympathetic and sympathetic ganglia, prevented both responses. This inhibition of the vegetative nervous system by tetraethyl ammonium persisted for only thirty to forty minutes. Because the effect of tetraethyl ammonium, though more complete than that of atropine, is of brief duration, it is not recommended for treatment of hypersensitive carotid sinus syndrome of this type.

We are indebted to Dr. E. C. Vonder Heide, Parke, Davis & Company, Detroit, Mich., for a generous supply of tetraethyl ammonium chloride (Etamon).

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LABORATORY METHODS

AN APPARATUS FOR CONTINUOUS RECORDING OF THE VOLUME OF EXPIRED AIR

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VARIOUS methods are available for recording the respiratory minute volume, most common of which is the collection of expired air in a spirometer or Douglas bag. Obviously there are a number of limitations to such a procedure. We therefore sought an apparatus which would give a continuous record, provide exact information from minute to minute, require practically no attention, and be simply and sturdily constructed. Such a unit with these characteristics has been in use in these laboratories for the past three years. It has been especially valuable in quantitative studies comparing respiratory stimulation[1] and depression[2] by drugs. Both man and dog have been employed with the same machine. Although not yet tried, respiration of unanesthetized dogs could easily be measured by utilizing an appropriate mask. A very brief description of the method has been given previously.[3] Details of construction are herein reported.

APPARATUS

The design of the collecting apparatus is shown in Fig. 1. It consists of two Krogh-type spirometers attached by an overhead cord and pulleys to counterbalance each other. These spirometers are connected to standard one-half inch galvanized pipes to carry the expired air. Four solenoid valves are located in the pipes, two in front of and two behind each spirometer. The solenoids are closed except when activated. The circuit is so arranged that one front valve and the opposite rear solenoid are opened simultaneously, and vice versa. This enables one spirometer to empty while the other is being filled with expired air. Mercury switches are situated above the tanks, contact with which controls the operation of the solenoids. Once contact is made, the mercury switch is inactivated until the tank trips the opposite one. This prevents any possible error, since connections are made to an automatic counter and to a signal magnet for recording on paper. The diagram of the wiring is given in Fig. 2.

In close proximity to the subject or test animal are two valves. Their construction is seen in Fig. 3 and resembles that described by Wright.[4] Each valve consists of a glass tube surrounded by a small pool of mercury on which rests a lightweight inverted glass cup. One valve allows air to enter for inspiration, while the other transmits expired air to the collecting device. The dead air space is small to prevent undesired effects on normal respiration. All tubing is at least 13 mm. inside diameter to allow air to pass freely. The valves operate easily and are of sufficient size to eliminate points of resistance. We have tried

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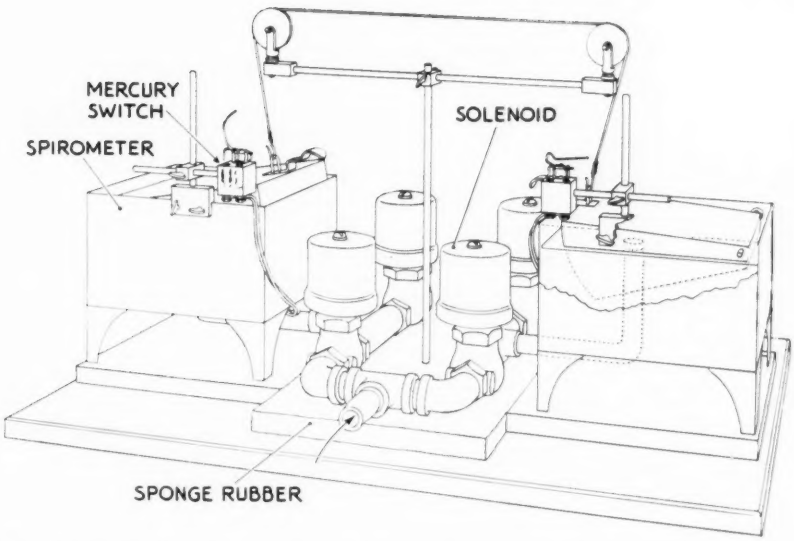

Fig. 1.—Apparatus for continuously collecting and measuring the volume of expired air.

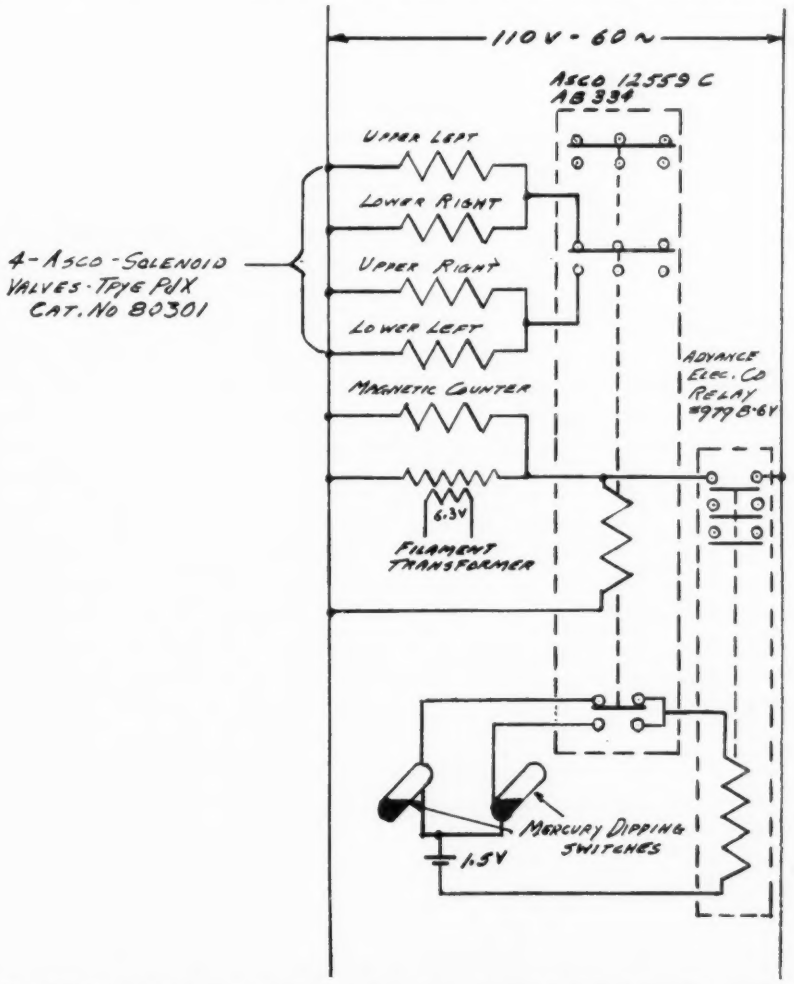

Fig. 2.—Wiring diagram of collecting apparatus, switch box, and countersystem.

various rubber-flap valves, including those described by Chambers and associates,[5] but in our experience they tend to leak, particularly during strong respiratory movements. A light bulb with reflector placed close to the expiratory valve provides sufficient heat to prevent condensation of moisture.

The exact resistance to flow of air was measured for different rates and depths of respiration. To do this, a water manometer was connected to a side arm of the tube leading to the subject. For comparison, measurements were made also with a standard basal metabolism machine and a 177-liter Tissot spirometer. The results with the three types of apparatus were quite comparable.

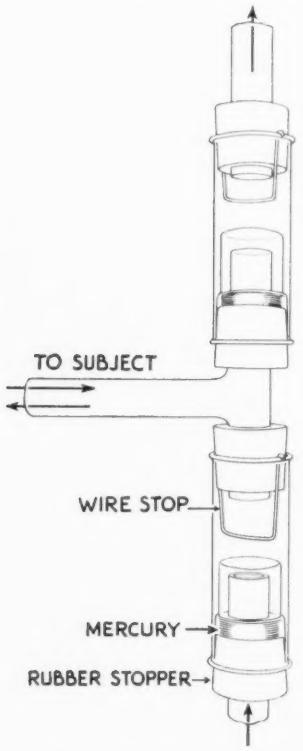

Fig. 3.—Respiration valves which direct expired air to collecting apparatus but prevent rebreathing.

With normal respiration, our machine showed values of −5 mm. water and +25 mm. water on inspiration and expiration, respectively. This compared with −7 mm. and +15 mm. for the Tissot spirometer, and −3 mm. and +2 mm. for the basal metabolism apparatus. When the rate and depth of respiration were augmented, resistance increased greatly with all three devices. Expiratory pressures increased to 70 to 90 mm. water, while there was a decrease of inspiratory pressures to −30 to −60 mm. water. There was no difference between the three methods.

Calibration of the spirometers may be done by a 100 c.c. syringe connected to the animal side of the inspiratory-expiratory valves. By adjusting the

length of the overhead cord, the capacity of the spirometers may be varied. The capacity of the two spirometers is made identical by proper location of the mercury switches. In our experience, 500 c.c. volume changes usually have been employed. Calibration values were determined using different rates of pulsation with the syringe. At rates of 30 to 60 pumps per minute, the error proved to be less than 1 per cent. For instance, ten fillings of each spirometer gave values of 764.5 ± 3.91 c.c. for the left spirometer and 755.5 ± 3.98 c.c. for the right tank, at a rate of 50 pumps per minute. At extremely slow rates of air flow, leakage does occur in the inspiratory-expiratory valve system. This is equally true with rubber-flap valves. However, these rates of air flow have no counterpart in the respiration of human beings or of dogs.

METHOD

Human subjects use a mouthpiece such as supplied with the usual metabolism apparatus. The mouthpiece is connected to the inspiratory-expiratory valves which in turn are attached to the spirometers. About five minutes are usually required for the subject or patient to become accustomed to the procedure and for respiration to stabilize. Subsequently, a recording or measuring period of ten minutes is sufficient, but a much longer duration is easily tolerated. This procedure may be repeated at regular intervals to test the prolonged action of such drugs as morphine.

In our experience, sodium phenobarbital is a good anesthetic for respiratory studies in dogs. Owing to its long action, respiration will remain unchanged for hours. This is a distinct advantage in measuring the stimulating or depressing action of any drug.

This apparatus should be easily adaptable to other types of respiratory studies. In case inspired gases other than ordinary air were needed, it would be necessary only to connect a supply of the desired gas or mixture of gases to the tube on the inspiratory valve.

SUMMARY

An apparatus is described which continuously records the volume of expired air. Essentially it consists of two spirometers with appropriate valves. While one spirometer fills with expired air, the other tank empties. At each filling, registration is made by both a signal magnet and an automatic counter.

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A MICROMETHOD FOR THE DETERMINATION OF THE ALBUMIN-GLOBULIN RATIO IN GUINEA PIG SERUM

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WHILE conducting certain studies on the sera of guinea pigs it was found desirable to determine the albumin-globulin ratio of these sera. Since the methods employed generally require the withdrawal by cardiac puncture of considerable amounts of blood, a micromethod was developed requiring only one-tenth of a milliliter of serum. The blood was obtained by making an incision perpendicular to and across the marginal vein of the ear and gently expressing the blood into a capillary tube. The technique of bleeding is described by Ecker, Hiatt, and Barr.[1]

METHODS

The micromethod is based on the methanol precipitation of the globulins in human serum as employed by Pillemer and Hutchinson.[2] The conditions were standardized in such a manner that the results obtained by the micromethanol precipitation technique agreed with those secured by the electrophoretic analysis of the same sera.

It was noted that at 0 to −5° C. the serum albumin remained soluble at a concentration of 42.5 per cent methanol, pH range of 5.3 to 5.5 (before addition of methanol), and ionic strength of 0.015. Under these conditions all the globulins were precipitated.

Table I. Comparison of the Albumin-Globulin Ratios of Guinea Pig Sera by Electrophoretic Analysis and by Methanol Precipitation

	Guinea pig*									
	1	2	3	4	5	6	7	8	9	10
Electrophoresis										
Gm. albumin per 100 c.c.	3.15	2.64	2.86	2.96	3.42	3.24	2.39	1.98	2.36	2.58
Gm. globulin per 100 c.c.	3.02	2.95	2.70	3.11	3.19	2.60	2.72	2.47	2.98	1.87
Albumin-globulin ratio	1.04	.89	1.06	.95	1.07	1.25	.88	.80	.79	1.38
A/G Micromethod										
Gm. albumin per 100 c.c.	3.17	2.62	2.69	2.89	3.35	3.25	2.36	1.90	2.44	2.49
Gm. globulin per 100 c.c.	3.00	2.97	2.87	3.18	3.26	2.59	2.75	2.55	2.90	1.96
Albumin-globulin ratio	1.05	.88	.94	.91	1.03	1.26	.86	.74	.84	1.27
Per cent deviation of AG ratio	.96	1.12	11.32	4.21	3.73	.80	2.27	7.50	6.34	7.60
Average deviation, 4.6%										

*Guinea pigs 1 through 6, normal guinea pigs; 7 and 8, bled by cardiac puncture for three successive days before getting serum sample; 9, post-partum guinea pig; 10, 4-week-old guinea pig.

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A comparison of the albumin-globulin ratios obtained electrophoretically and by the micromethod is found in Table I.

The results obtained indicate that the two methods agree closely, the average deviation being less than 5 per cent.

As an additional proof of the value of the micromethod, the globulins of 8 ml. of pooled guinea pig sera were precipitated at the same dilution and under the same conditions as employed in the microtechnique. The globulin precipitate was washed once and dissolved in a veronal buffer (pH, 8.6; μ, 0.1) for electrophoretic analysis. The supernatant albumin was lyophilized to dryness and also dissolved in veronal buffer.

The patterns of the two samples showed that the albumin fraction contained less than 2 per cent globulins, while the globulin fraction contained 7.7 per cent of albumin, most of which can be attributed to occlusion.

MATERIALS

Methanol Reagent.—Chemically pure methanol, 607 ml., are added with mixing to 393 ml. of distilled water. This mixture is cooled to 0° C. and made up to 1 liter with cold methanol.

Acetate Buffer (pH, 5.4; μ, 0.05).—Sodium acetate $3H_2O$, 6.8 Gm., and 1 N acetic acid, 10.6 ml., are diluted to 1 liter with distilled water. The pH of the solution should be between 5.3 and 5.5.

PROCEDURE

1. Place 2.9 ml. of acetate buffer in a 5 ml. volumetric flask and to this buffer add 0.1 ml. of the test serum with a volumetric pipette.

2. With a volumetric pipette remove 2 ml. of this 1:30 serum and transfer it to a 15 ml. conical centrifuge tube. Blow the small amount remaining in the tip of the pipette back into the 5 ml. flask so that exactly 1 ml. is left.

3. Add 1 ml. of the acetate buffer to the centrifuge tube and fill the volumetric flask to the 5 ml. mark with the buffer.

4. Cool the buffered serum in the centrifuge tube to 1° C. Add 7 ml. of the methanol reagent cooled to 0 to −5° C. Stir well, stopper, and allow to stand for thirty minutes at 0 to −5° C. During this period the globulins will precipitate, leaving the albumin in solution. The suspension is then centrifuged for thirty minutes at 2,000 revolutions per minute in a refrigerated centrifuge at 0 to −5° C.

5. Withdraw a 4 ml. aliquot sample of the supernatant with a volumetric pipette, transfer it to a digestion flask, and determine the nitrogen content.

6. A 4 ml. aliquot sample from the 5 ml. volumetric flask is then withdrawn and transferred to a digestion flask for a determination of its nitrogen content. From this analysis the total serum protein content is obtained. Nitrogen determinations are made according to the Pregl and Grant[3] technique with two blanks, one of which is made up of the same proportions as the albumin supernatant (3 parts buffer and 7 parts methanol), and the other consisting of 4 ml. of buffer.

CALCULATIONS

15/4 × mg. N in supernate = Gm. albumin per 100 ml. of serum
15/4 × mg. N in total protein sample = Gm. of total protein per 100 ml. of serum

$$\frac{\text{Gm. albumin N per 100 ml. of serum}}{\text{Gm. total protein N per 100 ml. of serum}} = \text{Per cent albumin}$$

Gm. total protein N per 100 ml. of serum - Gm. albumin N per 100 ml. of serum = Gm. globulin N per 100 ml. of serum
100 per cent - Per cent albumin = Per cent globulin

$$\frac{\text{Per cent albumin}}{\text{Per cent globulin}} = \text{Albumin-globulin ratio.}$$

SUMMARY

A method is presented which gives the albumin-globulin ratio in as small a quantity as 0.1 ml. of guinea pig serum. Values obtained by this technique compare favorably with those obtained electrophoretically. The conditions found here apply to guinea pig serum. Extension of the method to the sera of man and of other animals requires a readjustment of the conditions for precipitation.

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SCHLESINGER'S TEST FOR UROBILIN IN THE PRESENCE OF RIBOFLAVIN AND OTHER FLUORESCENT COMPOUNDS

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IN THE past, urinary fluorescence has been noted occasionally following the ingestion of acriflavine,[8] eosin,[18] and fluorescein.[5] With the recent widespread use of vitamin preparations green urinary fluorescence due to riboflavin is often found which interferes with the green fluorescence of urobilin produced in Schlesinger's zinc acetate-alcohol test. Such interference may be troublesome in cases of hepatic diseases in which urobilinuria is an important sign and, at the same time, vitamin B medication is an accepted treatment.

Ehrlich's test for urobilinogen, as recommended by Watson,[20-22] is generally preferred and is not influenced by fluorescent compounds with the exception of acriflavine.[15] The orange color or precipitate of the latter can be prevented by the addition of sodium acetate, according to Watson. Schlesinger's test, however, still has its merits, namely, in cases of old urine specimens where urobilinogen has been oxidized to urobilin, in determinations on urine of jaundiced patients where biliverdin formed by the hydrochloric acid of Ehrlich's reagent may mask the red urobilinogen reaction, in cases of albuminuria where protein interferes with Ehrlich's test,[16] and whenever a confirmation of Ehrlich's test is desired.

The following methods allow the performance of Schlesinger's test in the presence of riboflavin and other dyes exhibiting green fluorescence which may occur in urine, and tests for their identification are given.

TECHNIQUE OF PROCEDURES

*I. Schlesinger's Test in the Presence of Riboflavin**

Principle.—Urobilin is extracted with chloroform in which riboflavin is insoluble and the chloroform extract treated with zinc acetate and alcohol.

Method.—Ten cubic centimeters of urine, 10 c.c. of chloroform, 10 drops of concentrated HCl, and 2 drops of tincture of iodine are shaken and centrifuged. The chloroform layer is drawn off, mixed with half its volume of alcohol, about 0.2 Gm. of zinc acetate, and 1 drop of concentrated NH_4OH, and filtered. The filtrate is viewed through the height of the fluid column with strong side light against a background of black velvet for the presence of green fluorescence.

Comment.—The extraction of urobilin is not complete. Bilirubin is partially extracted and may exhibit a reddish fluorescence as first observed by Auché[2] and later investigated by Dhéré.[4] This red fluorescence, however, is generally too weak to interfere.

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*The products used were: riboflavin, Harris Laboratories, St. Louis, Mo.; acriflavine, Abbott Laboratories, North Chicago, Ill.; fluorescein, Merck & Co., Inc., Rahway, N. J.; eosin and erythrosin, National Aniline and Chemical Co., New York, N. Y.; mercurochrome, Hynson, Westcott & Dunning, Inc., Baltimore, Md.

*II. Schlesinger's Test in the Presence of Acriflavine**

Principle.—Acriflavine is rendered insoluble in chloroform by treatment with $NaNO_2$ in acid solution, resulting in a purple color reaction.

Method.—Ten cubic centimeters of urine are treated with 10 drops of concentrated HCl and 10 drops of 5 per cent $NaNO_2$ and the test completed as under I.

Comment.—An investigation into the nature of the purple color reaction which may be due to formation of a nitroso compound or to simple oxidation has not been attempted.

*III. Schlesinger's Test in the Presence of Fluorescein, Eosin, Erythrosin, and Mercurochrome**

Principle.—Urinary pigments are adsorbed on cotton in a Tweed column and urobilin is eluted with ammonia and alcohol.

Method.—A glass tube 20 by 1.5 cm. with tapered end (the lower end of a 100 c.c. burette is suitable) is stuffed moderately tightly with absorbent cotton to a height of 18 cm.; 10 c.c. of urine mixed with 10 drops of concentrated HCl and 20 drops of bromine water are poured on the column and allowed to soak into the cotton, followed by 1 c.c. of concentrated NH_4OH and 6 c.c. of 95 per cent alcohol. Two cubic centimeters of filtrate are slowly collected; 10 c.c. of alcohol and 3 drops of concentrated NH_4OH are added. Half of this mixture is filtered without and the other half filtered with the addition of about 0.3 Gm. zinc acetate. Fluorescence of the latter filtrate indicates the presence of urobilin, while the former serves as a control and may show a faint fluorescence due to fluorescein derivatives which were not completely retained by the cotton.

Comment.—The procedure is based on Arata's test[1] for the artificial coloring of food. Since urobilin is partly adsorbed[10] and incompletely eluted the test is not very sensitive and a weak fluorescence is evidence of increased urobilin. Bilirubin is retained in the cotton and thus separated from urobilin. Eosin is best adsorbed and fluorescein least, necessitating its conversion to eosin by bromine prior to adsorption. In order to insure sufficient retention of these dyes the pinkish or orange color ring appearing on elution should not move further than about two-thirds down the column.

IV. Spectroscopic Test of Urobilin and Fluorescent Dyes in Urine

Spectroscopic Detection of Urobilin in the Presence of Fluorescent Dyes.—

Principle.—A urinary chloroform extract free of flavins is treated with zinc acetate, alcohol, and acid whereby the spectrum of acid urobilin is produced while fluorescein derivatives do not give any absorption in acid solution.

Method.—The procedure is the same as under I. Five cubic centimeters of the final solution are acidified with 3 drops of concentrated HCl, filtered, and examined in a suitable layer with a wave length spectroscope† for the absorption of acid urobilin at 504 to 478 millimicrons.

See footnote on p. 1503.

†The grating spectroscope of Schmidt and Haensch, Berlin, was used.

Comment.—In the presence of much urobilin the usual Schlesinger filtrate may be used, but the absorption band is not so sharply defined. The spectroscopic test is far less sensitive than the fluorescence test.

SPECTROSCOPIC DETECTION OF FLUORESCENT DYES IN THE PRESENCE OF UROBILIN.—

Principle.—The urine is extracted with amyl alcohol, diluted with 95 per cent alcohol, and rendered alkaline. The comparatively weak absorption band of urobilin does not interfere as a rule.

Method.—Ten cubic centimeters of urine (or a larger volume) acidified with 4 drops of concentrated HCl are shaken with 5 cubic centimeter of amyl alcohol and centrifuged. The amyl alcohol is drawn off, mixed with an equal volume of 95 per cent alcohol and 3 drops of concentrated NH_4OH, and diluted with more alcohol if necessary. The solution is examined with the spectroscope for the absorption bands given in Table I.

TABLE I. SCHEME FOR PRELIMINARY IDENTIFICATION OF FLUORESCENT DYES IN URINE

COMPOUNDS	URINE OF YELLOWISH COLOR; STRONG YELLOW-GREEN FLUORESCENCE IN UV LIGHT	URINE OF PINKISH COLOR; DULL MAUVE FLUORESCENCE IN UV LIGHT	REMARKS
Riboflavin	Not extractable with ether or chloroform; spectroscopic: darkening of right side of spectrum up to about 500 mμ		
Acriflavine	Purple color reaction with $NaNO_2$ and acid; spectroscopic: similar to riboflavin		
Fluorescein	Conversion to eosin with bromine and acid; spectroscopic: 512 – 491 mμ		
Eosin		Adsorption on cotton; spectroscopic: 537–521 mμ	Filtering acidified urine through cotton in Gooch crucible
Mercurochrome		Test for mercury; spectroscopic: 534–511 mμ	Gettler's test[6] in urine, concentrated if necessary.[7]
Erythrosin		Weak fluorescence; spectroscopic: 528–502 mμ	Identification by absorption curve or biologic test.[3]

Comment.—The method of Discombe[5] may be used but it requires larger amounts of urine. Urobilin up to concentrations of 200 mg. per cent in terms of urobilinogen did not interfere when the dye concentrations were 1 mg. per cent. As noted from Table I, the absorption bands of eosin, mercurochrome, and erythrosin are very similar and it is doubtful whether spectroscopy alone will be sufficiently accurate for their differentiation.

DISCUSSION

The excretion of riboflavin after vitamin B medication depends on the dose of riboflavin, which may vary from 5 to 50 mg. per day,[11] on urinary concentration, and on the excretory capacity of the kidneys. The fluorescence

in concentrated specimens may be noticed easily under ordinary lighting conditions or may be so weak that it can be seen only when the filtered urine is examined with strong side light. But even weak concentrations are revealed instantly in filtered ultraviolet light by the brilliant yellow-green fluorescence which is superimposed on the gray-white fluorescence of normal urine.[13] Since treatment with vitamin B complex is advocated also in psychoses,[19] fluorescent urines are frequently found in patients in mental institutions.

There are only three reports on urinary fluorescence due to compounds other than riboflavin. Goldschmidt[8] found fluorescence caused by acriflavine (trypaflavin) which is listed in *The Dispensatory of the United States of America*[23] and Gutman's *Modern Drug Encyclopedia and Therapeutic Index*[9] as an external and internal disinfectant and, at one time, was used as a gall bladder antiseptic. Schumm[18] observed a fluorescent urine due to eosin, the source of which could not be determined. Discombe[5] found fluorescein in urine after ingestion of a proprietary drug. As fluorescein is used increasingly as a diagnostic aid, for example for the determination of the circulation time,[14] for the examination of the adequacy of peripheral blood vessels,[12] and for visualizing gastric ulcers during gastroscopy,[17] one may expect to find fluorescein in urine more often.

The author noted several cases of urinary fluorescence due to acriflavine following medication, two cases due to mercurochrome subsequent to bladder irrigation, and three cases due to fluorescein of undetermined origin. Recently, an eosinlike dye was found in urine following the swallowing of a lipstick by a mental patient. According to information received from the Food and Drug Administration* this dye most probably was rhodamine B, commonly used for coloring lipsticks. Another fluorescent urine on extraction yielded a dye which showed some characteristics of erythrosin but could not be identified definitely due to insufficient material. Erythrosin, the only red coal tar dye permitted for coloring food, is presumably ingested frequently but, due to its weak fluorescence and the small quantities excreted, has probably escaped notice.

The scant reports in the literature do not necessarily indicate that urinary fluorescence is not being encountered from time to time by laboratory workers and, if present in Schlesinger filtrates, it may be wrongly interpreted as urobilinuria. For such occasions it was thought helpful to refer to Table I which presents a simple scheme for the preliminary identification of fluorescent dyes which may occur in urine.

SUMMARY

1. A modified Schlesinger test has been described allowing the separation of urobilin from riboflavin whose green fluorescence in urine following vitamin B medication interferes with that of the urobilin-zinc compound.

2. Modifications of Schlesinger's test in the presence of fluorescent dyes occasionally present in urine, such as acriflavine and fluorescein and derivatives, have been described.

3. Tests for identification of fluorescent dyes in urine have been given.

*I wish to express my thanks to G. Robert Clark, Acting Chief, Cosmetic Division, Food and Drug Administration, for his helpful information.

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PROCEEDINGS OF THE CENTRAL SOCIETY FOR CLINICAL RESEARCH

Twentieth Annual Meeting

Chicago, Ill., Oct. 31 and Nov. 1, 1947

TO BE READ BY TITLE—CONCLUDED

51. CONTINUOUS OBSERVATIONS OF THE ARTERIAL OXYGEN SATURATION AT REST AND DURING EXERCISE IN CONGENITAL HEART DISEASE

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Analysis of arterial samples from twenty resting persons with congenital heart disease has been used as a reference point for continuous measurements of the arterial oxygen saturation with the Milliken compensated circuit oximeter during exercise and other procedures. Nineteen normal volunteers were studied as controls. Observations have been made while the individuals breathed pure oxygen, with and without positive pressure up to four inches (10.2 cm.) of water, while they stood upright, and while they walked on a power-driven treadmill at 1.7 miles per hour.

In normal persons at rest, oximeter readings indicated that the arterial saturation was increased by from 1 to 5 percentage points (mean, 2.7) in an average time of 1.3 minutes when pure oxygen was breathed. Addition of positive pressure to the oxygen supply caused no further change in the oximeter reading.

In resting patients with cyanotic types of congenital heart disease the arterial oxygen saturation averaged 71 per cent, ranging from 45 to 91 per cent saturation. Oximeter readings were increased from 2.0 to 16.5 percentage points (mean, 6.2) in an average time of 3.0 minutes when these patients breathed pure oxygen. When the oxygen was given with positive pressure, oximeter readings increased, on the average, an additional 1.6 percentage points.

The normal group showed no significant change in their arterial oxygen saturation when they assumed the upright position or when they exercised by walking on a horizontal treadmill at 1.7 miles per hour for five minutes. The oximeter readings of persons with cyanotic types of congenital cardiac defects decreased, on the average, 2.4 percentage points when they stood upright, and decreases ranging from 3.5 to 19.0 percentage points (mean, 10.9) occurred when they walked on the treadmill at 1.7 miles per hour for an average time of 3.5 minutes.

Simultaneous arterial samples and oximeter readings obtained both at rest and during exercise in a smaller series of patients indicated that the saturation

changes recorded by the oximeter were less than the changes found by the van Slyke analysis of arterial blood. Nevertheless, use of the oximeter in these tests has proved a valuable objective adjunct in assessing the degree of dysfunction in such patients both before and after surgical corrective procedures have been attempted.

52. A TWELVE-YEAR STUDY OF A PARTICULAR GROUP OF HYPERTENSIVE PATIENTS PRE- AND POSTSYMPATHECTOMY WITH REFERENCE TO RENAL PATHOLOGY

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In the past twelve years twenty-five cases of essential hypertension were studied jointly by the medical and surgical services of a university hospital. A part of this study is reported in this paper, the correlation of vasomotor tests with the anatomic changes found in renal biopsies (six) or at autopsy (four).

Selection of a particular group of patients for consideration of surgical therapy was made in the hope that eventually the surgical treatment of hypertension might be completely and properly evaluated. These patients were those whose course was rapidly progressive despite vigorous, nonsurgical therapy. The immediate prognosis in all instances was considered to be good; the ultimate prognosis, however, was always poor.

The same surgeon (Dr. Loyal Davis) performed the several types of sympathectomy, adding thereby to the uniformity of the results.

During the period of this study numerous tests for vasomotor lability were introduced as an aid in the selection of cases suitable for sympathectomy. The following tests were employed: (1) resting blood pressure, (2) sodium amytal test, (3) cold pressor test, (4) continuous caudal anesthesia, (5) carbon dioxide sensitivity, (6) reaction to tetraethylammonium chloride, and (7) a new test, the vertavis test. Most were repeated after sympathectomy and some were observed in the interval between operations in cases where a thoracolumbar sympathectomy was performed. These tests have been compared with the anatomic changes found and the clinical course to date. During the period of observation, none of the tests showed a high degree of correlation with reference to these two factors. Some of the patients reported on here have been followed continuously for twelve years after sympathectomy. Proper case selection for the surgical treatment of hypertension, we feel, is still most difficult, if not impossible, at this time. It is our impression that patients not responding to thiocyanate therapy are unsuitable for sympathectomy.

53. THE EFFECTS OF A DIET OF RICE AND FRUIT JUICES UPON THE BLOOD PRESSURE OF HYPERSENSITIVE INDIVIDUALS*

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(Introduced by Edward H. Reinhard, M.D., St. Louis, Mo.)

Attempts were made to evaluate the use of a diet composed of rice, fruit juices, and vitamin supplements in the treatment of arterial hypertension, since others have reported beneficial effects from this and other low salt diets.

*This study was supported by a grant-in-aid from the United States Public Health Service.

Six patients, five of whom showed essentially normal renal function, were studied extensively, control periods of approximately one month in the hospital being observed. These patients suffered from arterial hypertension in varying degrees of severity, two being in the malignant stage and two in severe and two in moderately severe stages. Patients were placed on the diet for three to five weeks.

In four patients the average diastolic blood pressure changed less than 10 mm. Hg. In the other two it fell 11 and 13 mm. Hg while on the diet but did not return to the control level when a normal diet was substituted.

An attempt was made to determine whether the limitation of salt intake imposed by the special diet played a part in the observed effects upon blood pressure. Serum chloride levels fell in all cases, but the serum sodium concentration did not. Addition of sodium chloride to the diet produced no consistent effects upon blood pressure. Weight was lost by four patients.

It is concluded that the effects of the rice and fruit juice diet upon blood pressure were minimal and inconsequential. Effects which were observed could not be correlated with changes in the salt content of the diet.

54. FURTHER STUDIES WITH THE HISTAMINE TEST FOR PHEOCHROMOCYTOMA

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Previously we reported on the intravenous injection of small amounts of histamine base as a test for distinguishing the syndrome of pheochromocytoma from other clinical conditions. At that time, the injection of 0.05 or 0.025 mg. of histamine base produced the clinical syndrome, including hypertension and the characteristic symptoms, in three patients known to have pheochromocytoma. After surgical removal of the tumor, the injection of the same amount of histamine produced no paroxysm of hypertension or symptoms. As a result, we tentatively suggested this method as a test for the clinical syndrome of pheochromocytoma.

We now wish to present two additional cases of pheochromocytoma. In one of these, paroxysmal hypertension was present and the result of the histamine test was positive. The test was of proved value in arrival at the diagnosis. In the second patient, persistent hypertension with edema of the optic discs was present. The result of the histamine test in this case was regarded as negative because the increase in blood pressure after the injection of histamine did not exceed the increase in blood pressure during the cold pressor test. The failure to secure a positive result in this case of persistent hypertension may possibly be explained on the basis that the tumor contained only a relatively small amount of epinephrine (62 mg.) and that it was probably incapable of secreting sudden explosive charges of epinephrine because it was secreting epinephrine continuously.

A total of four cases, however, can now be reported in which the injection of histamine into patients with pheochromocytoma will produce an "attack" exactly similar to that of which they complain, in which marked elevation of the blood pressure occurs.

Tetraethylammonium chloride failed to produce a paroxysm of hypertension on two occasions in the patient known to have a pheochromocytoma.

The histamine test has been carried out on 200 patients with hypertension and fifty other patients with other clinical conditions. No untoward effects have been experienced by any of these patients. This would seem to indicate that the test is not hazardous.

55. DIRECT MEASUREMENT OF BLOOD PRESSURE IN THE RADIAL AND FEMORAL ARTERIES DURING AND AFTER TILTING

ROBERT S. GREEN, M.D. (BY INVITATION), ARNOLD IGLAUER, M.D., AND JOHNSON MCGUIRE, M.D., CINCINNATI, OHIO

Direct tracings of radial and femoral artery pressure were obtained by a Statham Strain Gauge during and after tilting at a moderate rate between the 20 degree erect and 45 degree head-down position. The recordings from the radial artery were made with the arm maintained in a horizontal plane throughout the tilt.

Blood pressure in the radial artery invariably rose during the tilt from the head-up to the head-down position. In normal subjects, this increase of blood pressure (average, 19 mm. systolic/14 mm. diastolic) was followed by a fall, the blood pressure returning within eight to eighteen seconds to approximately the level of the head-up position. During the return tilt to the head-up position, the blood pressure always fell. In normal subjects, the pressure decreased (average, 14 mm. systolic/13 mm. diastolic) to a level less than in the initial erect position and returned within eight to eighteen seconds to approximately the initial level in the erect position.

The blood pressure in the femoral artery fell during the tilt from the head-up to the head-down position, and in the normal subjects then fell further, reaching a stabilized level in eight to eighteen seconds. During the return to the head-up position, the femoral blood pressure always increased, and in normal subjects this increase was followed by a further rise, the blood pressure reaching the range of the starting level in eight to eighteen seconds.

The increase in radial artery pressure and the decrease in femoral artery pressure during the tilt from the erect to the head-down position and the opposite changes during the return tilt indicated that these alterations are secondary to gravity. Since these primary changes were followed in both arteries by a fall of blood pressure in the head-down position and by an elevation of blood pressure in the erect position, it is believed that these secondary responses were neurogenic. The changes in the radial artery are interpreted as a reflection of the changes in the large arteries arising from the aortic arch, since the arm was maintained at the level of the arch throughout the tilt. The neurogenic responses tended to maintain the blood pressure at a stable level at the region of the aortic arch. This suggests that the purpose of these reflex adjustments of blood pressure is to protect the brain and perhaps the heart from adverse pressure changes.

56. CLINICAL AND EXPERIMENTAL STUDIES ON THE EFFECTS OF DIATHERMY ON BLOOD FLOW

KHALIL G. WAKIM, M.D., PH.D., J. F. HERRICK, PH.D. (BY INVITATION), AND JEROME GERSTEN, M.D. (BY INVITATION), ROCHESTER, MINN.

In normal individuals as well as in patients (none of whom had any impairment in circulation), the blood flow in the upper and lower extremities was studied by the use of the plethysmograph with a compensating spirometer recorder before and for thirty to thirty-five minutes after application of diathermy for half an hour. Within five to fifteen minutes after diathermy, in seven of the twenty-five patients, the blood flow in the treated extremity increased more than 100 per cent. The increase in blood flow in the twenty-five patients ranged from 39 to 268 per cent, with an average increase of 71 per cent. At the same time the untreated extremities showed an average increase of 5 per

cent, but in nine of the twenty-five patients the untreated extremity showed an initial decrease in blood flow. At the end of thirty minutes after stopping the diathermy treatment, the blood flow in the treated extremities still showed an average increase of 56 per cent over the control value. There was no significant difference between the results obtained on patients and on normal individuals.

In dogs anesthetized with pentobarbital sodium, the blood flow was studied, by means of a bubble flowmeter in the femoral artery or vein, before, during, and after application of diathermy to the thigh for a period of twenty minutes. During the application of diathermy the significant increases over the control blood flow ranged from 16 to 209 per cent, with an average increase of 69 per cent. In four of the seventeen experiments slight decreases of 24 per cent, 18 per cent, 5 per cent, and 5 per cent, respectively, from the control blood flow occurred. After treatment with diathermy was stopped, the significant increases in blood flow ranged from 16 to 142 per cent, with an average increase of 64 per cent. In two of the seventeen experiments there was a slight decrease in blood flow after treatment with diathermy was stopped; namely, 15 and 10 per cent, respectively. In one experiment blood flow did not change after cessation of diathermy. Subcutaneous and muscle temperatures were taken in the dog by means of needle thermocouples and were recorded galvanometrically. The increase in muscle temperature at the end of the application of diathermy ranged from 0.9 to 9° C., with an average increase of 4.0° C.

57. A QUANTITATIVE HYPOTHERMAL METHOD FOR PRODUCTION OF LOCAL INJURY TO TISSUE

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Local necrosis of tissue can be produced rapidly and quantitatively by use of an instrument cooled to a low temperature by internal expansion of carbon dioxide or flow of cold liquids. The cooling element of the instrument is flat and circular, so that when it is applied to the surface of an organ or tissue, cylindrical lesions are produced. The diameters of the lesions can be varied from 2 to 25 mm. by using cooling elements of comparable diameters. The depths of lesions can be varied from 1 to 13 mm. by varying the time of contact of the cooling element with the tissue. Volumes of injured tissue can be accurately determined by measurement. Lesions of identical size and location may be reproduced in successive experiments. Lesions have been produced in cartilage, bone, skin, skeletal muscle, vascular walls, heart, liver, kidneys, and brain of rabbits. Necrosis is uniform throughout the lesions and there is a sharp line of demarcation between nonviable and normal tissue at the periphery. Suppuration does not occur and hemorrhage is never found except around blood vessels in lesions of the brain. Organs and tissues can be progressively and selectively destroyed in a manner which is not possible by other methods such as cauterization, vascular ligation, or surgical excision.

58. ANEURYSMS OF THE CORONARY ARTERIES DUE TO POLYARTERITIS NODOSA OCCURRING IN AN INFANT: REPORT OF A CASE WITH CORONARY ARTERY THROMBOSIS AND MYOCARDIAL INFARCTION

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(Introduced by W. B. Wood, Jr., M.D.)

Since polyarteritis nodosa occurs infrequently in infancy and aneurysms of the coronary arteries with coronary artery thrombosis and myocardial infarction represent a rare complication, the following instance involving a 15-month-old infant is reported.

The patient, a white female infant, had been well until the age of 1 year, at which time because of rash and fever of three days' duration the patient was admitted to the Children's Hospital, St. Louis. The infant appeared acutely but not critically ill. The temperature was 39° C. In addition to the rash which was believed to represent an erythema multiforme, there was marked conjunctivitis and pharyngitis. The tympanic membranes were edematous. The remainder of the physical examination revealed nothing pertinent. The blood showed a leucocyte count of 16,000 with normal differential and a hemoglobin of 9 Gm. per cent. Tuberculin and Kline tests were negative and no pathogens were identified in urine, stool, and throat cultures. The rash began to fade on the fourth hospital day but the infant continued to appear ill. The temperature remained between 38 and 40° C. until the tenth day and then slowly subsided. The patient was discharged as improved on the seventeenth hospital day.

Three months later, on Sept. 16, 1946, the patient was readmitted because of dyspnea, orthopnea, anorexia, and fatigue on slight exertion of three weeks' duration. She appeared acutely and chronically ill. The heart and respiratory rates were rapid and the heart sounds of poor quality. Cardiac enlargement and pulmonary congestion were demonstrated by fluoroscopy. Blood examination revealed a leucocyte count of 10,400 per cubic millimeter, a moderate lymphocytosis, and hemoglobin of 10 Gm. per cent. Several hours after admission the infant suddenly became rigid, made some convulsive movements, and ceased to breathe. Emergency therapy revived the patient only temporarily and she expired five hours after admission.

At autopsy the heart presented the most significant and interesting findings. It weighed 80 grams (normal, 50 grams). The left coronary artery had a normal ostium but the lumen was narrowed to pin-point size at the bifurcation. The anterior descending branch of the left coronary artery presented a fusiform enlargement which began 3 mm. from its origin and extended for a distance of 15 millimeters. It measured 10 mm. in its greatest diameter. An adherent thrombus measuring 30 mm. and completely occluding the lumen was found in this branch of the left coronary artery. A portion of the thrombus occluded the circumflex branch at its origin. For most of its course the circumflex branch had a thickened wall and narrowed lumen. Fifteen millimeters from its origin the right coronary artery presented an aneurysmal dilatation measuring 18 mm. in length and 6 mm. in diameter. The wall of the artery was thickened for the initial 35 mm. of its course.

The left ventricle was dilated and the wall thickened (8 mm.) at the base but thin (3 mm.) at the apex. There were evidences of recent and remote infarction in the anterior portion of the interventricular septum and the anterior and apical portions of the left ventricle. The right ventricular wall was hypertrophied (6 mm.). Microscopically vascular changes representing healing and

healed polyarteritis nodosa were observed. Diffuse fibrosis and numerous areas of fresh infarction were found in sections of the anterior and apical portions of the myocardium of the left ventricle and the anterior portion of the septum.

59. ORAL MERCURIAL DIURETICS IN AMBULATORY PATIENTS WITH CONGESTIVE HEART FAILURE

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(INTRODUCED BY JOHNSON MCGUIRE, M.D.)

Previous reports of the use of oral mercurial diuretics in the treatment of the decompensated cardiac patient have stressed their use during hospitalization. We are presenting a preliminary report of the use of orally administered mercurial diuretics upon ambulatory patients seen in the Cardiac Clinic of the Out-Patient Dispensary at the Cincinnati General Hospital.

Fifty-six patients with cardiac edema have been treated from three to six months with orally administered mercurial diuretics, using Mercuzanthin.* All patients had previously received mercurials parenterally. The type of heart disease in twenty-six was hypertension; in fourteen, rheumatic; in eight, arteriosclerotic; in seven syphilitic; and in one, congenital.

The dosage schedule has varied, but a majority of patients now under treatment are taking two or three tablets daily. A minority take three tablets two or three times weekly.

To determine the adequacy of renal function, measurement of the blood urea nitrogen and a complete urinalysis were performed on each patient prior to initiation of therapy. Repeat blood urea nitrogen determinations were performed monthly, and urinalysis was done bimonthly.

Clinical improvement was gauged objectively by weight loss, decrease of edema and râles, increase in vital capacity, decrease in venous pressure; and subjectively by decrease in effort dyspnea, orthopnea, and paroxysmal nocturnal dyspnea.

Of the fifty-six patients treated, forty are currently receiving therapy. Thirty-five are considered improved and five unimproved. In nine patients the treatment has been discontinued for various reasons; these include four instances of painful stomatitis and two of diarrhea. Seven patients have died during the course of therapy; however, a review of the clinical records and autopsy protocols does not suggest that death was the result of mercurial toxicity. No renal complications have been observed. Stomatitis has appeared in eight patients, sufficiently severe to require discontinuance of therapy in four. Abdominal symptoms consisting of nausea, vomiting, abdominal cramps, and diarrhea have appeared in eight patients; treatment was stopped in three. Therapy was later restarted and continued successfully in one of these.

Our conclusion is that oral mercurial diuretics frequently can effectively replace parenteral mercurials in the ambulatory patient and that such replacement has materially reduced the daily patient load in this Cardiac Clinic.

*Mercuzanthin (Distributed by Campbell Products, Inc., New York, N. Y. Each tablet is equivalent to 0.030 Gm. mercury and 0.027 Gm. anhydrous theophylline).

60. PHYSIOLOGIC ADJUSTMENTS TO SUDDEN CHANGES IN ENVIRONMENTAL TEMPERATURE AND HUMIDITY OF HEALTHY YOUNG SUBJECTS AND CARDIAC PATIENTS

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Physiologic adjustments of healthy young male medical students to sudden changes in environmental temperature and humidity have been studied. The subjects remained for one hour in a comfortable environment (76° F. with relative humidities of 30, 60, or 80 per cent) and then entered a hot moist environment of (98.5° and 66 per cent relative humidity) for one or two hours. Then they re-entered the comfortable room and remained for one hour.

When subjects entered a hot moist environment from a comfortable environment, their mean skin temperature rose quickly and approximated its maximum value in less than ten minutes. The rectal temperature decreased with the first ten minutes and then slowly increased. The pulse rate and systolic pressure increased slightly while the diastolic pressure decreased slightly.

When they entered an environment conditioned for comfort from a hot moist environment, the mean skin temperature fell rapidly but it fell less rapidly when the relative humidity was high (80 per cent). The rectal temperature increased within the first ten minutes and then slowly decreased. The blood pressure showed no significant variation, although large fluctuations were occasionally observed. The pulse rate decreased but it decreased less when the relative humidity was high (80 per cent). In addition, the subjective sensation of comfort was obtained earlier and the evaporative weight loss per gram of moisture present on the skin and in the union suit was less when the relative humidity was high.

Patients with cardiovascular impairment (arteriosclerotic heart disease, angina pectoris following coronary infarction, hypertensive heart disease, rheumatic heart) are now being studied under comparable experimental conditions. In addition to the observations mentioned, determination of plasma volume, vital capacity, basal metabolic rate, and the effects of Master's two-step tests were made to define the degree of circulation impairment. Preliminary data on these impaired older patients reveal that their thermal adjustments are accomplished with equal ease and no clinically significant greater strain on the cardiovascular system occurs than that shown by the healthy young medical students.

61. A CARDIAC FACTOR WHICH INCREASES CORONARY FLOW UNDER SYMPATHETIC STIMULATION

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Stimulation of the stellate ganglion and cardiac nerves was shown by Gregg and Shipley to increase coronary blood flow. The exact mechanism of this change remains unknown.

In a further study of this problem in the anesthetized open-chest dog, blood flow in the left anterior descending or left circumflex coronary arteries was

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measured by a recording rotameter and a phasic flow meter either under the normal pulsatile aortic pressure or under a constant perfusion pressure. Flow measurements were made before, during, and after stimulation of the left cardiac accelerator nerves both before and after section from the stellate ganglion.

When both mean and phasic flows were recorded simultaneously, mean flow often increased before the phasic flow meter revealed any change in the resistance to flow as indicated by a constant rate of diastolic flow. Inspection of aortic and intraventricular pressure curves showed a marked shortening of cardiac contraction, as described by Wiggers and Katz, following cardiac nerve stimulation and during the action of adrenalin. In addition to the systolic shortening, our records show a marked increase in the rate of diastolic relaxation.

Actual flow measurements and calculations of increases in absolute lengths of diastole during minute intervals (from intraventricular curves) show that these alterations in systolic and diastolic time ratios account for from 10 to 30 per cent increases in flow, in addition to further increases due to decreased resistance to flow. In this manner, the heart, by its increased velocity of contraction and relaxation, reduces the time of intramyocardial vascular compression during systole, thus substituting longer periods of the high diastolic flow for long periods of low systolic flow despite moderate increases in heart rate. Since the highest rate of coronary blood flow occurs in diastole, it follows that any lengthening of the total diastolic time in a given minute must increase mean blood flow.

These studies are being continued in an effort to evaluate other mechanical factors responsible for increasing coronary flow after nerve stimulation such as changing heart size, intramyocardial tension, and increased vascular emptying resulting from the augmented vigor of contraction.

62. THE VENTRICULAR GRADIENT IN HYPERTHYROID CONDITIONS

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(Introduced by Johnson McGuire, M.D.)

In a study of the effect of hyperthyroidism and of thyroid substance on the heart, the electrocardiograms of a group of cases were analyzed by study of their ventricular gradients. These cases were of two groups, the first composed of fourteen patients with hyperthyroidism and the second of eleven normal subjects to whom 6 grains of thyroid substance were administered daily. All patients studied were free of complicating diseases and all had electrocardiograms that were normal except for a few instances where transient abnormalities of cardiac rhythm were noted.

In the group with hyperthyroidism all of the fourteen patients showed an increase in the magnitude of their ventricular gradient as compared with control electrocardiograms taken after remission of the disease induced by surgery or by thiouracil. This increase averaged 39 per cent, with a range from 4 to 100 per cent. In addition, seven of this group showed a significant clockwise shift in the direction of the ventricular gradient averaging 26 degrees, with a range of plus 16 to plus 48 degrees. The other seven in this group showed no significant shift in the direction of the gradient.

In the group receiving thyroid extract, all of the eleven subjects showed an increase in magnitude of the ventricular gradient. This magnitude averaged 30 per cent, with a range from 7 to 73 per cent. None of these subjects showed any significant shift in direction of the gradient.

In both groups increase in heart rate was a common occurrence, and values obtained for magnitude and direction of ventricular gradients were corrected for changes in heart rate.

It is suggested that the increased magnitude of the ventricular gradient is a reflection of the increased work of the heart. It is also suggested that the shift in direction of the gradient in the hyperthyroid group may indicate myocardial strain since this finding has been previously noted in cases of hypertension and of acute glomerular nephritis.

63. THE PRECORDIAL ELECTROCARDIOGRAM IN LEFT VENTRICULAR HYPERTROPHY: A STUDY OF AUTOPSIED CASES

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An analysis has been made of the Wilson precordial leads in eighty-four pathologically proved cases of left ventricular hypertrophy without associated myocardial infarction or appreciable gross fibrosis. This comprises all cases occurring within a five-year period in which satisfactory electrocardiograms were obtained within three months of death except for thirty-seven cases of bundle branch block.

By means of a Cambridge device, measurements of the time elapsing from the onset of QRS to the nadir of Q, to peak of R, to nadir of S, and to end of QRS were made in three complexes of Leads $V_{5,6}$. The average amplitude of the individual components of the QRS, the contour and level of the RST segment, and the amplitude and direction of the T waves were determined in each precordial lead. Similar measurements were made in a control series of fifty-two cases in which the hearts were normal at autopsy, and in a second group of fifty young male adults with normal hearts by physical and roentgen examination.

Differentiation between pattern of left ventricular hypertrophy and of normals could not be made from the amplitude of the R waves in V_5 and V_6 alone since the average values were only slightly higher in the former.

In 98.0 per cent of normals the time interval from the onset of QRS to peak of R was less than .05 second. In 93.1 per cent of normals the time interval from onset of the R wave to its peak was less than .04 second. For purposes of classification, measurements equalling or exceeding these figures were regarded as abnormal. A Q-R of .048-.049 and/or an R of .038-.039 second were regarded as borderline and values below this, as normal. Of the eighty-four patients with left ventricular hypertrophy, thirty-four (or 40.5 per cent) showed an abnormal Q-R and/or R duration. The average cardiac weight in this group was 610 grams. Eight (or 9.5 per cent) showed borderline values and the cardiac weight averaged 569 grams. Forty-two cases (or 50 per cent) showed normal values, with an average cardiac weight of 536 grams. There was a general trend toward increasing duration of Q-R or R with increasing cardiac weight, but many individual exceptions were encountered.

In eight cases where R or RST-T findings in precordial Leads V_5 and V_6 were borderline or normal, abnormalities referable to left ventricular hypertrophy were found either in V_7 or in the unipolar extremity leads.

In seven cases the electrocardiogram showed nonspecific abnormalities and in four it was regarded as normal.

64. THE FORM OF THE ELECTROCARDIOGRAM IN ANTEROSEPTAL MYOCARDIAL INFARCTION COMPLICATED BY RIGHT AND LEFT BUNDLE BRANCH BLOCK

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Clinical and experimental studies have shown that although the electrocardiographic diagnosis of anterior infarction can be made in the presence of right bundle branch block, the characteristic signs of myocardial infarction are obscured by left bundle branch block unless the infarct involves the interventricular septum. Observations have been made in two patients in whom anteroseptal myocardial infarction was complicated first by transient right bundle branch block and then by transient left bundle branch block. This permitted an unusual opportunity to compare in the same individuals the standard and precordial electrocardiograms in normal conduction and both types of intraventricular block in the presence of myocardial infarction.

The first patient was a man aged 50 years, who had a typical acute coronary thrombosis without antecedent cardiac symptoms. When right bundle branch block was present the standard electrocardiograms showed large S waves in all leads, while records from the right precordium displayed prominent Q waves and large late R deflections. When left branch block was present, Lead I showed no negative deflections and the large Q waves and late R peaks previously recorded from the right, now appeared over the left precordium. The characteristic signs of infarction were confined to Leads CR_2 and CR_3 when normal conduction was present. Partial heart block and complete localization of the infarct and the extension into the septum were confirmed at postmortem examination.

The second patient was a man aged 52 years who had possible mild angina pectoris for several months before the acute infarction occurred. Large, late R waves were again recorded from the right precordium when the right banch of the His bundle was blocked and large Q or QS waves appeared in all of the precordial leads. When left branch block developed, tracings taken from the left precordium resembled those recorded over the right precordium during the period of right branch block; prominent waves appeared in Lead I. The precordial electrocardiogram during normal conduction suggested that the infarct was anteroseptal in location. The clinical course was complicated by paroxysmal auricular fibrillation but a satisfactory recovery occurred.

In anterior myocardial infarction complicated by intraventricular block, the ventricular complexes recorded from the precordial area overlying the ventricle which is activated late are very similar in form in both right and left bundle branch block in those cases in which the myocardial lesion also involves the interventricular septum.

65. ELECTROCARDIOGRAPHIC CHANGES AFTER ELECTRIC SHOCK TREATMENTS

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In persons with suspected heart disease, but normal electrocardiograms, it has been possible to produce abnormalities in the electrocardiograms by partial oxygen deprivation (Levy) or by measured exercise (Masters). The cases presented here were patients with definite electrocardiographic abnormalities who were subjected to the extreme anoxemia and violent exercise

produced by psychiatric electric shock treatments. To the surprise of the writer, the electrocardiograms assumed a more normal pattern for a few minutes after the treatment.

Case 1 was a man 47 years of age who had suffered a coronary occlusion four years previously. Before treatment the electrocardoigram showed low to flat T waves in Lead I and slightly inverted T waves in Lead II. Immediately after treatment, the inverted T waves became flat.

Case 2 was a 59-year-old woman who had hypertension and a partial hemiplegia. An electrocardiogram showed sharply inverted T waves in Leads I and CF IV and V. Immediately after electric shock treatment the previously inverted T waves had become flat to upright. One minute after treatment they were slightly inverted, two minutes after treatment they were more inverted, and three minutes after treatment they were just about the same as before treatment.

Case 3 was a 41-year-old man who had had a coronary occlusion two months before electric shock treatment. The electrocardiogram showed sharply inverted T waves and deep Q waves in Leads II and III. Immediately after treatment there was a partial cardiac arrest, the rate falling to 20 per minute. The T waves remained inverted. However, the QRS complexes became upright to diphasic.

Case 4 was a 73-year-old woman whose physical examination was negative and who gave no history of cardiac disease. Her electrocardiogram before treatment showed inversion of the T waves in Lead CF IV. Immediately after treatment the T waves became upright. They remained upright for three minutes, then became flat and remained flat for another ten minutes, and at the end of twenty minutes they had become inverted and had resumed their pretreatment configuration.

Case 5 was a 40-year-old woman with no history of cardiac disease. The T waves were found to be low to flat in Lead I and flat in Lead CF IV. Immediately after treatment the T waves in Lead I had become upright. They remained upright for three minutes and then resumed their previous flat condition.

The small number of cases presented do not warrant any conclusions. It may be said that the increase in amplitude or return to normal of the T waves are the physiologic response to exercise, such as one sees in normal athletic hearts. However, this is not the response in the Masters test, and the foregoing may be considered an extreme exaggeration of this test.

As compared with the tests of Masters and Levy, these results seem paradoxical.

66. THE EFFECT OF A SINGLE INSPIRATION IN LEAD III AND LEAD CF IV OF THE HUMAN ELECTROCARDIOGRAM

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The effect on the electrocardiogram of a single deep inspiration during Leads III and CF IV was studied in order to (1) ascertain the frequency and type of respiratory changes produced; (2) determine if any correlation exists between these changes and the presence or absence of heart disease; and (3) determine the influence of digitalis on these respiratory variations. Two thousand three hundred seven consecutive tracings were obtained for this study.

An initial increase in rate followed by slowing of various degrees frequently occurred, but in very few instances was it possible to demonstrate changes in the auriculoventricular conduction with the changes in rate. Digitalis was associated with the most striking changes in rhythm including usually a shift of the pacemaker from the sinoauricular node toward the auriculoventricular node, but the depression of the pacemaker also occurred in the absence of digitalis.

Numerous changes including variations in contour of P waves, QRS complexes, and T waves in Lead III were almost as frequent in normal as in abnormal records. The QRS complexes usually returned to their original configuration very shortly after the T waves assumed their previous form.

In Lead III of normal records there was a decided tendency for inspiration to result in increasing positivity of all deflections, but in Lead CF IV there was a tendency for inspiration to cause increasing negativity of all deflections. In abnormal electrocardiograms, except in those of patients with acute myocardial infarction or in those of subjects under digitalis therapy, inspiration tended to increase the positivity of all deflections in Lead III. In the patients who received digitalis, or who had acute myocardial infarction, inspiration tended to increase the negativity of the T waves in Lead III with an opposite effect on the other deflections. The frequency of T-wave changes in Lead CF IV with inspiration detracts from the significance of the T wave in the fourth lead in the interpretation of borderline records.

Respiratory effects on the electrocardiogram include changes in axis, in T waves, and in cardiac mechanism. It has been suggested that the axis changes are probably the result of extracardiac factors, that T-wave changes are probably the result of intracardiac factors, and that variations in cardiac mechanism are the result of nervous reflexes, chiefly vagal.

67. AN UNUSUAL VAGOVAGAL REFLEX TYPE OF ADAMS-STOKES SYNDROME

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(INTRODUCED BY MAURICE HARDGROVE, M.D.)

A patient with a vagovagal type of reflex Adams-Stokes syndrome associated with an anterior traction diverticulum of the esophagus was studied. Spontaneous attacks occurred when cardiospasm caused irritation of the diverticulum through dilatation of the esophagus, and the vagal reflex sensitivity was increased by digitalization. Minor clinical attacks and electrocardiographic evidence of cardiac slowing or arrest occurred in the digitalized patient on distention of a balloon inserted in the esophagus to the level of the diverticulum, but not above or below that level. Right carotid sinus stimulation caused the same phenomenon. Atropine abolished these findings through interruption of the vagal reflex. Withdrawal of digitalis resulted in decreased sensitivity of the carotid sinus and vagovagal reflex irritability to a point where the clinical and electrocardiographic findings were not obtained. Intravenous administration of 0.8 mg. of digitoxin caused re-establishment of the carotid sinus and vagovagal reflex irritability within four hours.

This is the second case, known to us, of Adams-Stokes syndrome due to vagovagal reflex associated with an esophageal diverticulum and the first in which the summation role of digitalis in producing the syndrome has been reported. This summation role of digitalis in reflex types of cardioinhibitory Adams-Stokes syndrome seems worthy of note.

68. THE VARIABILITY OF THE FASTING NOCTURNAL GASTRIC SECRETION IN NORMAL INDIVIDUALS AND IN PATIENTS WITH DUODENAL ULCER

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The fasting nocturnal gastric secretion was measured in thirty-three normal individuals and in thirty-two patients with active duodenal ulcer. The average twelve-hour volume, concentration of hydrochloric acid, and total output of acid were significantly higher in patients with duodenal ulcer than in normal individuals. In the normal group, the volume averaged 581 ml.; the free acidity, 29 clinical units; and the total output of acid, 661 milligrams. In the duodenal ulcer series, the volume averaged 1,004 ml.; the free acidity, 61 clinical units; and the total output of acid, 2,242 milligrams. Similarly, the average hourly secretion was persistently higher in patients with duodenal ulcer than in normal individuals. Wide individual variations were observed in both groups.

In addition, considerable spontaneous fluctuation in the hourly and total night secretion was noted in the same individual. Thus, in the normal subjects, the average percentage variations in the volume, free acidity, and total output in the same individual were 24, 44, and 47 per cent, respectively. In patients with duodenal ulcer the average spontaneous variation was: volume, 17 per cent; free acidity, 24 per cent; and total hydrochloric acid output, 23 per cent. The volumes in both groups were significantly higher during the first six hours of the night as compared with the last six hours. There was no important difference in the average concentration of free hydrochloric acid during the various periods of the night in the normal subjects, whereas in the duodenal ulcer series, the output of acid was significantly lower during the last three hours of the night than at any other time.

These variations occurring spontaneously in the same individuals must be taken into account in the proper evaluation of procedures utilized for the purpose of reducing gastric secretion.

69. ATROPHIC GASTRITIS AND MALNUTRITION

LEONIDAS H. BERRY, M.D., CHICAGO, ILL.

Most of the conceptions and investigations into the causes of gastritis in the past have been concerned with extrinsic factors and local irritation. It is probably true that next to the skin the organ which is subjected to the most rigid abuse from man's external environment is the stomach. Today investigators show a definite trend of interest in the role of intrinsic factors in gastric disease. Psychosomatic factors are being emphasized.

Our studies of patients with chronic atrophic gastritis show the etiologic importance of malnutrition and indicate that most of the chronic atrophic gastritis coming under our observation is probably primary rather than inflammatory. Malnutrition not due to disease, most commonly but not invariably, follows an economic pattern. This fact was very striking in our studies.

The analysis of data on 706 dyspeptic patients gastroscoped 1,000 times during four years immediately preceding World War II showed some striking differences when compared with 297 patients with dyspepsia gastroscoped 364 times during the four war years. In the "prewar" group (1937 to 1942) 53.6

per cent of the total chronic gastritis seen was the atrophic type. The remaining 46 per cent was about equally distributed between the chronic superficial and chronic hypertrophic types. The "wartime" group (1942 to 1946) showed a considerable decline in chronic atrophic gastritis (from 53.6 per cent to 18.5 per cent) and a corresponding rise in the superficial and hypertrophic types. Thus chronic "atrophic gastritis" was three times higher in the "prewar" group. What is the significance of this variation in distribution? Rather extensive laboratory studies were done. A large percentage of the "prewar" group had dietary questionnaire and food habit studies. Ninety per cent of the "prewar" group were clinic patients, unemployed from two to four and up to eight years prior to examination. Nutrition diaries clearly showed that most of these patients existed on diets inadequate in quantity and quality of protective foods to maintain normal nutrition. There were evidences of low or borderline plasma ascorbic acid levels, red blood cell counts, hemoglobin values, spongy gums, underweight, and other evidences of subclinical states of undernutrition. Symptomatically those patients with so-called atrophic gastritis were fatigued easily and had attacks of weakness in addition to abdominal distress.

As employment improved the ratio of patients on relief to those gainfully employed was reversed. Therapeutic studies (reported in another communication) making use of liver extract, vitamin B complex, and improved diets showed striking symptomatic improvement and convincing disappearance of atrophy. These facts contribute further evidence to the etiologic relationship of malnutrition to so-called chronic "atrophic gastritis."

70. THE EFFECT OF CHOLINE, METHIONINE, AND LOW FAT DIET ON THE LIFE EXPECTANCY OF PATIENTS WITH CIRRHOSIS OF THE LIVER

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(INTRODUCED BY CARL HARFORD, M.D.)

In a previous communication the following regimen for the treatment of cirrhosis was described:

1. Diet	
Protein	90-100 grams
Fat (chiefly vegetable)	50 grams
Carbohydrate	Sufficient for patient's caloric needs
2. Drugs	
Choline hydrochloride	1-8 grams
Methionine (1 quart skimmed milk)	0.9 grams
3. Vitamins	
A	10,000 U. S. P. units
D	800 U. S. P. units
4. Abstinence from alcohol	

This regimen was based on two assumptions: (1) that fatty changes in the liver ordinarily precede the more characteristic anatomic changes of cirrhosis; (2) that fatty changes in the liver are often dietary in origin, especially in the alcoholic patient, and are comparable to the changes observed in experimental animals placed on deficient diets. The present report summarizes the clinical experiences of the past six years, during which time some 224 patients with cirrhosis of the liver have been studied. The fate of these patients is compared with that of a similar group of 311 patients treated in the same hospital under similar circumstances prior to the use of the present therapeutic regimen.

The fate of the two groups of patients, as of March 1, 1947, is as follows:

	EXPERIMENTAL		CONTROL	
	NUMBER	PER CENT	NUMBER	PER CENT
Dead	168	77.1	265	85.2
Living	44	20.2	7	2.3
Fate unknown	12	2.7	39	12.5
Total	224	100.0	311	100.0

Comparison of the survivors in the two groups permits no conclusion regarding the merits of the therapeutic regimen since the period of observation of the experimental group has been too brief. The period of survival thus far from the onset of initial symptoms varies from 13.0 to 63.2 months for the experimental series as compared with 71.5 to 167.5 months for the control series. The average period of survival is 33.0 months and 112.2 months, respectively.

It is not possible to utilize all the patients known to be dead in the two series since some were thought to have died from causes not directly related to their liver disease. Adequate data regarding the onset of symptoms were not available in other instances. The experimental series is thus reduced to 138 patients and the control group to 196 cases.

If one plots the percentage of patients surviving at any given time after the onset of the initial symptoms, the curves for the experimental and control groups superimpose one upon the other. Similiar curves comparing the survival rate after the onset of ascites, jaundice, or hemorrhage also reveal no difference between the experimental and control groups.

It is concluded, therefore, that the therapeutic regimen as carried out was without effect upon the life expectancy of patients with cirrhosis.

71. HEPATIC FUNCTION IN INFECTIOUS MONONUCLEOSIS

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(INTRODUCED BY OVID O. MEYER, M.D.)

Liver function tests were performed one or more times in forty-eight consecutive cases during the course of infectious mononucleosis. The presence of infectious mononucleosis was established with reasonable certainty according to existing criteria.

The following tests were made one or more times on each patient with the results indicated: icterus index in 43 patients, abnormal values obtained in 16; urine urobilinogen in 33, elevated values in 20; cephalin cholesterol flocculation test in 47, value of 3 plus or more in 37; thymol turbidity in 41, value of 4 units or more in 16; prothrombin time in 41, levels below 75 per cent in 5; 2 mg. per kilogram twenty-minute bromsulfalein test in 21, 10 per cent or more retention in 1. Certain tests were performed three or more times in several patients. Of 24 patients who had the cephalin cholesterol flocculation test three or more times, 23 (96 per cent) attained a value of 3 plus or more at least once. Eleven of twenty patients who had thymol turbidity tests three or more times attained a level of 4 units or more at least once. For the patients who had a series of observations, the average period from onset of disease to last abnormal value for Hanger's test was 34.7 days, and for thymol turbidity test, 38.9 days. Persistence of symptoms was noted for a variable period after the liver function tests performed were normal in seven of fifteen patients with adequate follow-up. The tests to date have not revealed a significant correlation between the heterophile antibody reaction and Hanger or thymol turbidity test.

72. EXPERIENCES WITH NEEDLE BIOPSY OF THE LIVER IN PATIENTS WITH MALIGNANT HEPATIC NEOPLASM

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CINCINNATI, OHIO

Needle biopsy of the liver was performed with the Vim-Silverman needle in forty-seven patients with proved malignant neoplasm of the liver. The transpleural approach was used in thirty-six of the patients and the abdominal approach in eleven. The tumor was primary in ten and secondary in thirty-seven. Neoplasm was demonstrated in the tissue obtained from thirty-nine of the group. The tumor was missed in eight biopsies, in three of which the specimen obtained was inadequate for microscopic examination.

The liver was palpated as nodular or irregular in twenty-nine of the patients and as smooth in seventeen. In one patient it was not palpable. The clinical diagnosis of malignant neoplasm of the liver was confirmed by means of the biopsy in thirty cases. In nine patients, three with primary hepatic tumor, carcinoma was not suspected prior to the biopsy. In sixteen of the twenty-nine patients with nodular liver, and in twelve of the twenty with smooth liver, the biopsy was the first means by which the presence of carcinoma was definitely established.

Liver biopsy proved particularly valuable in patients in whom the presence of carcinoma was not previously established and in whom "liver function" studies, especially the cephalin cholesterol flocculation test, suggested the presence of hepatitis.

Needle biopsy of the liver is a surprisingly valuable adjunct in the diagnosis of primary and secondary neoplasm of the liver and not infrequently may be the earliest or the sole means of establishing the diagnosis.

73. INFECTIOUS AND TOXIC HEPATITIS: PATHOLOGIC AND CLINICAL MANIFESTATIONS

HANS POPPER, M.D., FREDERICK STEIGMANN, M.D., MURRAY FRANKLIN, M.D. (BY INVITATION), AND LEO F. NARUT, M.D. (BY INVITATION), CHICAGO, ILL.

Studies in the Army Institute of Pathology on fatal cases of infectious (viral hepatitis revealed a characteristic morphologic picture. Investigation of fatal cases of acute primary hepatitis observed in a civilian hospital during eighteen years showed in less than one-half a morphologic picture similar to the one seen predominantly in military personnel; these cases were considered to be infectious in origin. Since in many of the other cases a hepatotoxic etiologic factor was present, these were tentatively designated as toxic hepatitis. The main morphologic differences were slower cell death, slighter mesenchymal involvement, outspoken zonal distribution, and occasionally predominant fatty changes in the toxic cases. Applying similar differentiating points to biopsy specimens of thirty-seven cases of acute primary nonfatal hepatitis, usually a differentiation between infectious and toxic hepatitis was possible. Subsequently a correlation with the apparent etiologic factors was found. More than one-half of the studied cases revealed a morphologic picture designated as toxic.

Based on this differentiation, we attempted to divide the cases of acute primary hepatitis by clinical and laboraotry methods. For instance, usually patients with toxic hepatitis are older and have a shorter prodromal period with

more intense gastrointestinal symptoms (vomiting and diarrhea) which continue even while the patient is in the hospital in contrast to infectious hepatitis in which the gastrointestinal symptoms decrease when jaundice appears. The patients with toxic hepatitis appears sicker, more feverish and dehydrated, are more often deeply icteric, and have tachycardia. Pruritis and dermatitis may be present. The spleen is more often felt. There is no clear-cut differentiation in the laboratory findings. In general, however, cephalin flocculation and thymol turbidity are more often negative and alkaline phosphatase and total cholesterol more often elevated in the toxic cases. In toxic hepatitis, the nonprotein nitrogen is often elevated and in the fatal cases icteric nephrosis is common, some of the patients dying in uremia. The dehydration in toxic hepatitis may be held responsible for the renal involvement just as dehydration has been considered an important pathogenetic factor in lower nephron nephrosis.

Definite establishment of the etiologic factors in acute hepatitis by virus studies might justify the thus far tentative designation of infectious and toxic hepatitis. The present findings suggest two different entities and that not every case of acute primary hepatitis in civilians is of infectious origin.

74. METHIONINE IN THE TREATMENT OF CHRONIC AND ACUTE HEPATITIS

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Encouraged by results of treatment of experimental nutritional cirrhosis by lipotropic substances, methionine, choline, or choline plus cystine were used as supplement in patients with liver cirrhosis. While the reports on such regimes are few and on small series of cases, most of them are rather optimistic.

In a comparative study of different lipotropic substances during the past two years, a group of ten patients with hepatic cirrhosis received, in addition to a high protein, high carbohydrate, high vitamin, and low fat diet, daily 5 Gm. of methionine orally. Seven of the patients had ascites; all with the exception of three were jaundiced; their symptoms varied from ten days to five years. They were treated in the hospital from ten days to five months. Three patients died during the first stay in the hospital and seven recovered and were discharged. Of these, two returned six and twelve months later, respectively, and died during the second admission. Of the remaining five, two are still ailing, while three have returned to their previous occupation, at which they have remained so far nine to twelve months. The jaundice disappeared in the recovered cases; the ascites decreased in two, and in three paracentesis is still occasionally necessary. The liver function improved in the surviving cases, especially the albumin/globulin ratio and the cephalin flocculation. Some improvement of liver function occurred even in two patients who later died.

Comparison of the results of this group with another without lipotropic supplement reveals a definite improvement both as to mortality and recovery. It should be considered that the variations in the therapeutic effects are not only due to different clinical stages, but also possibly to different types of cirrhosis.

The evaluation of therapeutic results in acute hepatitis is difficult because of the erractic course of the disease. It appeared therefore more revealing to study the influence of methionine upon the nitrogen balance in patients with acute infectious hepatitis. In four such patients no protein-sparing effect was found in experiments composed of several five-day periods. These results

speak against the existence of a ethionine deficiency in acute infectious hepatitis due to diversion of methionine from protein formation to lipotropic or other liver-protecting activity.

75. PROGNOSTIC VALUE OF BIOPSY IN CIRRHOSIS OF THE LIVER

William D. Davis, Jr., M.D., New Orleans, La.

(Introduced by Thomas Findley, M.D.)

Liver biopsies were accomplished in twenty-one patients with cirrhosis either by needle or surgical approach. Follow-up data were available in sixteen of these. In this paper an attempt has been made to determine the prognostic value of changes in the liver by correlating the degree of fatty change, fibrosis, necrosis, and inflammation in the liver sections with the response to therapy. The relation of ascites and the presence or absence of central veins was also noted. Treatment consisted of high carbohydrate (500 Gm.), high protein (200 Gm.), variable fat diet with supplements of brewer's yeast, vitamins, liver extract, and, in some instances, methionine or choline.

Of the group, eleven were considered to be decompensated and of these, regardless of the degree of fibrosis, necrosis, and inflammation, only those with rather severe fatty change showed significant improvement under treatment. In the compensated group microscopic changes were relatively minor and the clinical course was correspondingly good. In general, those patients with necrosis and inflammation showed better clinical and microscopic improvement than those with only fibrosis. The two patients with severe necrosis and inflammation without fatty change both died during the acute episode. Four other patients with similar changes plus considerable fatty infiltration have returned to useful life. In general, the patients with ascites showed relatively fewer central veins than those without ascites.

76. AN UNUSUAL CASE OF ESSENTIAL NORMAL CHOLESTEREMIC XANTHOMATOSIS

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Extensive biochemical, roentgenographic, electroencephalographic, and histologic studies have been made in the case of a 38-year-old white man with cutaneous xanthomatosis and grand mal, uncinate, and psychomotor seizures.

The initial manifestations of the disease appeared two and one-half years before. There was no history of seizures prior to the development of xanthomatosis and no family history of seizures.

Electroencephalograms showed diffuse cerebral disorder with some left-sided localization, and marked abnormalities were elicited following left carotid pressure which produced temporary asystole and faintness.

Total cholesterol, 196 mg.; free, 48 mg., cholesterol esters, 148 mg. per 100 ml. of blood.

Roentgenograms of the skull were normal. The long bones showed medullary thickening in the lower portion of the shafts of the femora and humeri but no punched-out areas. Biopsy of the femur showed xanthomatous involvement.

The most unusual feature of the case was the finding of scattered petechiae and small raised mahogany lesions over the limbs and trunk which tended to

appear in crops and fade in a few weeks. In addition, he showed classical chamois-colored lesions involving the lids and the entire scalp. Biopsy of one of the petechiae showed infiltrations of reticuloendothelial cells and eosinophiles around small blood vessels. Such lesions are extremely rare in adults with xanthomatosis of this type.

The patient suffered a pathologic fracture near the biopsy site after release from the hospital. His seizures have been satisfactorily controlled with dilantin sodium.

This case appears to represent essential xanthomatosis of the normal cholesteremic type (eosinophilic granuloma, eosinophilic xanthomatous granuloma) involving skin, bone, and dura, with eosinophilic granulomas involving the small blood vessels of the skin.

77. "COMBINED ANTACID" THERAPY IN PEPTIC ULCER

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CHICAGO, ILL.

The large number of antacids available for the treatment of peptic ulcer indicates that no one substance is of value in all cases. The disadvantages of some of the currently used antacids are acid rebound, alkalosis, too-transient neutralization, interference with normal gastric function, and unpalatability. Hence, the search for better antacids continues. Recently we have studied the antacid effect of a substance which has been prepared with the idea of combining the properties of currently used antacids but eliminating some of the untoward effects. This substance is a mixture of aluminum hydroxide, magnesium trisilicate, and purified gastric mucin.

Twenty-five patients with symptomatology and x-ray evidence of ulcer (twenty duodenal and five gastric) were chosen for this study. The fasting stomach was aspirated at fifteen-minute intervals for one hour before 0.5 mg. of histamine phosphate was injected subcutaneously. Following this, five further aspirations were done. On successive days, two tablets of the new preparation, of amphogel, or two Sippy tablets were chewed and swallowed following the fourth fasting aspiration and prior to the histamine injection. The free and total acidity was determined in all samples by titration with 0.1 N sodium hydroxide solution, using Töpfer's solution and phenolphthalein as indicators.

In 92 per cent of the cases, a variable antacid effect was obtained with all substances. In 55 per cent, the mucin mixture was more effective; in 27 per cent, less effective. In 18 per cent, they were equally effective. In 70 per cent of the cases, the mucin preparation was observed in the aspirated samples from fifteen to forty-five minutes longer than the other antacids. Recent gastroscopic and x-ray studies by Hardt confirmed this observation by demonstrating that the mucin preparation remained in the stomach longer. It was also noted that the mucin mixture was a more effective antacid when chewed than when allowed to dissolve in the mouth.

The preliminary antacid studies with this preparation which are now being supplemented by clinical observations suggest that this substance may prove a valuable addition to peptic ulcer therapy.

78. TRANSITION OF PANCREATIC EDEMA INTO PANCREATIC NECROSIS

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Pancreatic edema or pancreatic necrosis can be produced easily in the dog, but we have not been able, heretofore, to observe edema progress or change into necrosis. This has been achieved now by temporary clamping of the upper pancreatic artery in the presence of edema of the pancreas. It is felt that spasm of arteries may play a role in the development of pancreatitis.

79. BIOCHEMICAL FINDINGS IN ACETYLSALICYLATE THERAPY OF ACUTE RHEUMATIC FEVER

William S. Hoffman, M.D., Italo F. Volini, M.D., Mark Pomaranc, M.D. (By Invitation), and Catherine Nobe, B.A. (By Invitation)
Chicago, Ill.

Eighty patients with acute rheumatic fever were treated with high doses of aspirin combined in tablet form with colloidal aluminum hydroxide (Alasil, Wander). Preliminary tolerance studies had shown that the plasma salicylate levels obtained after single doses of the aspirin product were of the same order as those obtained with ordinary aspirin. But the gastrointestinal disturbances obtained in 13 per cent of the patients on high doses of ordinary aspirin did not occur with the aspirin containing aluminum hydroxide.

From 94 to 144 grains of aspirin daily (about 120 mg. per kilogram) usually produced plasma salicylate levels around 30 mg. per 100 milliliter. At this level, symptoms of salicylism often occurred with an accompanying irresponsibility and negativism. Many patients refused further to cooperate with the treatment and these had to have the dosage lowered. Thus in only forty-one patients was an average level of more than 25 mg. per 100 ml. maintained. The patients were treated until subsidence of clinical symptoms.

There was no great difference in the percentage of patients whose sedimentation rate subsided to normal, whether average salicylate level was above or below 25 mg. per 100 milliliter.

The plasma prothrombin levels were not much altered. They were reduced below 75 per cent of normal in only 33 per cent of the cases. In only one patient was the level low enough to produce bleeding, which was relieved by one single injection of vitamin K. There was no greater incidence of reduced prothrombin concentrations in the patients with high plasma salicylate concentration than in those with lower levels.

In almost all patients, a moderate reduction in serum bicarbonate occurred, usually associated with increased chloride concentrations and with slightly lowered sodium concentrations. The serum pH was usually normal. This might have been due to primary alkalosis produced by hyperventilation, but there was no marked hyperpnea, the urine was never alkaline, and clinical improvement occurred with bicarbonate. These findings along with those obtained with salicylate and chloride clearance studies pointed to the idea that salicylate and chloride compete for sodium in renal excretion and that when sodium is limited both are retained to produce a primary fixed acid acidosis.

80. TREATMENT OF SUBACUTE BACTERIAL ENDOCARDITIS WITH PENICILLIN IN OIL AND BEESWAX

WILLIAM S. HOFFMAN, M.D., ITALO F. VOLINI, M.D., AND J. R. HUGHES, M.D. (BY INVITATION), CHICAGO, ILL.

Eight patients with all the recognized clinical criteria for subacute bacterial endocarditis were treated with daily single injections of 2 ml. of hard Romansky formula penicillin (crystalline potassium penicillin in peanut oil and beeswax*) containing 600,000 units. Six of the patients had one or more positive blood cultures from which pure strains of *Streptoococcus viridans* were isolated. No bacteria could be isolated from the remaining two patients. The treatment was continued for forty-two days, the daily injections being given in alternate buttocks. All of these patients had histories and findings of an underlying rheumatic heart disease.

In these eight patients, the treatment was successful. Remission of fever and clinical symptoms occurred early in the treatment, in some cases during the first week. Cultures became negative in all cases, in some cases before the end of the first week, in no cases later than three weeks. Usually there was gain in weight and strentgh and ameliorization of anemia. One patient with right hemiplegia on admission from cerebral embolism had severe malnutrition and bedsores during the early part of the treatment, even though the cultures became negative. When her diet was supplemented with intravenous amino acids, glucose, and vitamins B and C, she improved rapidly. There were no urticarial reactions to the many injections. In one obese patient, the induration at the sites of injections required heat to alleviate pa in. In no other cases were there any significant local or general reactions. The penicillin sensitivity of the isolated bacteria ranged from 0.008 to 0.3 units per milliliter. Plasma penicillin levels ranged from 1 to 4 units at two hours to values of 0.03 to 0.125 at twenty-four hours. Only one zero level was obtained at twenty-four hours.

A ninth patient with subacute bacterial endocarditis from a focus in the bladder and posterior urethra had *Str. viridans* in the blood with a penicillin sensitivity of 4.15 units per milliliter. He was treated with daily injections of 3 ml. of Romansky penicillin (900,000 units) for thirty-four days without success. After a change to 500,000 units of aqueous penicillin every three hours intramuscularly, the patient still had a positive culture on the fourth day of the new treatment.

The use of Romansky formula penicillin for the treatment of subacute bacterial endocarditis is thus a convenient and successful method if the organism is not too penicillin-resistant.

81. TREATMENT OF LOBAR PNEUMONIA WITH PENICILLIN IN OIL AND BEESWAX

ITALO F. VOLINI, M.D., J. R. HUGHES, M.D. (BY INVITATION), J. R. PEFFER, M.D. (BY INVITATION), AND WILLIAM S. HOFFMAN, M.D., CHICAGO, ILL.

One hundred four consecutive patients entering the Cook County Hospital with lobar pneumonia were treated with daily injections of hard Romansky formula penicillin (crystalline potassium penicillin G in peanut oil and beeswax*). The daily dosage was 1 ml. containing 300,000 units. Seventy patients had typable pneumococci in the sputum; of the remaining thirty-four, some almost certainly had primary virus infections. Sixty-two per cent of all the

*Commercial Solvents Corp., New York, N. Y.

patients showed a satisfactory remission of fever and symptoms within seventy-two hours. Many of these showed this response after one injection. The percentage of good results was somewhat higher in the group of proved pneumococcal origin. There were four deaths, these occurring in patients with serious complications and of the older age groups. Many of the patients who responded slowly had handicapping complications, such as delirium tremens, chronic heart disease, chronic lung infections, and cirrhosis. There was only one urticarial reaction to the penicillin and no local abscesses.

Plasma penicillin concentrations obtained in twenty-five hospital control patients who were administered 300,000 units of the penicillin in oil and beeswax averaged 0.43 unit per milliliter at two hours, 0.11 at six hours, and 0.09 at twenty-four hours. There were three zero levels at twenty-four hours. In the 104 therapeutic cases, 122 determinations at twenty-four hours showed seven zero levels, the bulk of the values lying between 0.03 and 0.125 unit per ml., with an average of 0.10. At two hours the average level was 0.85 unit per milliliter. Since almost all pneumococci are sensitive to concentrations of 0.03 unit per milliliter or less, the levels obtained over the twenty-four hours with the single daily injections should have been therapeutically satisfactory.

The satisfactory results in over 50 per cent of the bacteriologically unproved cases of pneumonia suggests that penicillin therapy is indicated in all cases of pneumonia. However, if no satisfactory results are obtained in forty-eight hours, the case should be clinically re-evaluated.

82. LABORATORY AND CLINICAL OBSERVATIONS ON THE USE OF A NEW SULFONAMIDE—3,4-DIMETHYL-5-SULFANILAMIDO-ISOXAZOLE (NU 445)

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In vitro comparisons of the bacteriostatic activity of sulfadiazine and NU 445 were carried out with synthetic media to eliminate the polypeptide inhibitory factor. It was found that the two sulfa drugs studied were roughly comparable in action against *Escherichia coli*, gamma streptococcus of fecal origin, and hemolytic streptococcus. The acetylated forms of the drugs inhibited the growth of *Esch. coli* and gamma streptococcus but required slightly higher concentrations than the free drug. Folic acid was found greatly to inhibit the activity of both sulfadiazine and NU 445 against the gamma streptococcus but not against *Esch. coli.*

In twenty-four patients receiving NU 445 orally and parenterally in doses of 4 to 6 Gm. daily, the average blood level (fifty-three determinations) was 9.10 mg. per cent total and 6.36 mg. per cent free drug. About 30 per cent of the drug was therefore present in the N_4 conjugated form (acetylated), a figure considerably higher than for acetylated sulfadiazine or sulfamerazine as determined in this laboratory. In two instances in which NU 445 levels were determined on blood and spinal fluid from the same patient obtained the same day the spinal fluid levels were approximately one-half those of the blood levels.

Slightly more than one-half of the drug administered was recovered in the urine, about 35 per cent being present in the acetylated form. These figures closely approximate those obtained with sulfadiazine.

NU 445 was used clinically in 24 cases, including 6 patients with meningitis (meningococcal, 3; pneumococcal, 2; undetermined etiology, 1), 6 with urinary tract infections, 5 with respiratory infections, 2 patients with erysipelas, 1 with

scarlet fever, and 4 with postoperative infections. In 14 the drug was used alone and in 10 it was used in combination with penicillin and/or streptomycin. In no case was a patient's death thought to be due to failure of the drug to control the infection. Both patients with meningococcus meningitis treated with NU 445 alone recovered.

Reactions occurred in four cases, consisting of a generalized dermatitis in one, crystalluria and hematuria in one, and nausea and vomiting in two others. No local reaction resulted from repeated intramuscular injection of the drug (lithium salt) in 10 per cent solution.

83. CLINICAL STUDIES OF INSOLUBLE KETONES AS INTESTINAL ANTISEPTICS

PRELIMINARY REPORT

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Since the discovery of the properties of sulfaguanidine by Marshall, Bratton, White, and Litchfield in 1940, it has been shown that many other sulfonamide groups had similar intestinal antiseptic properties. The clinical advantage claimed for the use of compounds that are absorbed slightly or not at all from the intestine is the obviation of side effects.

Recently a new group of compounds was discovered that are absorbed very slowly from the intestine and yet demonstrate antibacterial activity. The group is derived from p-aminobenzene sulfinic acid (a series of insoluble ketones). Ketones Nu201 and Nu404 were studied. The toxicity values are expressed as a single oral dose of 20 Gm. per kilogram tolerated by white mice for ketone Nu201 and 15 Gm. per kilogram for ketone Nu404. We have administered the new agents to patients orally in doses of one tablet three times daily before meals. Ketone Nu201 was administered over a period from seven to twelve days and Nu404 from eleven to forty-nine days. The blood levels for Nu201 ranged from .08 to 1.2 mg. per milliliter and those for Nu404 from .09 to .8 mg. per milliliter.

In our experience Nu201 is apparently toxic to some patients.

Bacteriologic studies in the stools showed a reduction in the intestinal flora in the staphylococci and streptococci groups. The studies on the *Escherichia coli* group were inconclusive. Clinically, the patients experience a reduction in the number of stools per day, disappearance of abdominal cramping, and a decrease in the amount of blood in the stools. In general, our preliminary experience with Nu404 is more favorable. More work is needed to confirm and to extend our present findings.

84. THE COMPARATIVE EFFECT OF VARIOUS ALKALIS AND UREA UPON THE BLOOD CONCENTRATION, EXCRETION, AND CONJUGATION OF SULFONAMIDES WITH GLUCURONIC AND ACETIC ACID

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The most valuable sulfonamide adjuvant would be one which enhances glucuronic acid conjugation, depresses acetylation, and prevents crystalluria. Since sulfonamides conjugated with glucuronic acid are less likely to result in

uroliths and are more antibacterial than acetylated forms according to the literature, it seemed worth while to study the effect of various adjuvants on the conjugation of sulfa drugs with glucuronic and acetic acid.

Sulfadiazine was given orally to one patient and sulfamerazine to the other on alternate three-day periods for two months with various adjuvants. Sodium citrate and sodium lactate dosages were equivalent to 24 Gm. of sodium bicarbonate daily and the dosage of urea was 60 Gm. per day. Glucuronic acid and the sulfa fractions were determined in blood and urine.

Total sulfonamide blood concentrations were slightly reduced by the alkali adjuvants but not by urea. No significant effect of adjuvants on the percentage acetylated was demonstrated.

In most instances blood glucuronic acid was increased by sodium citrate and sodium lactate adjuvants but not by sodium bicarbonate, nor the sulfonamide alone. Urea depressed the blood concentration of glucuronic acid.

Urinary glucuronic acid was increased by both sulfadiazine and sulfamerazine either with or without adjuvants.

In general there were greater increases of urinary glucuronic acid after alkali adjuvants were administered than when urea or no adjuvant was given. These increases above controls, which were taken on days that adjuvants only were given, were calculated to represent equimolar conjugation with sulfonamide.

The percentage of urinary acetylated sulfonamide was less when the alkali adjuvants were given than when urea or no adjuvant was given.

Urea had no effect on blood concentration, excretion, or conjugation. Most of the ingested urea was recovered in the urine. Blood and urine urea nitrogen had returned almost to control levels twenty-four hours after stopping the drug.

The fact that sodium citrate and sodium lactate increase conjugation of sulfadiazine and sulfamerazine with glucuronic acid and decrease conjugation with acetic acid is an additional indication for the use of adjuvant alkali therapy with these drugs.

85. CRYSTALLURIA FOLLOWING SULFONAMIDE MIXTURES AND SULFONAMIDES PLUS ALKALI: A COMPARATIVE STUDY OF 400 CASES

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(INTRODUCED BY CHARLEY J. SMYTH, M.D.)

The use of sulfonamides is attended by the danger of renal complications. Lehr (1945) and Flippen (1946) showed that using a mixture of two sulfonamides markedly reduced crystalluria. This occurs because sulfonamide solutions are mutually soluble. Flippen demonstrated that using single sulfonamides, 12 Gm. of sodium bicarbonate are necessary to keep crystalluria incidence equal to that of sulfonamide mixtures.

This study compares the incidence of crystalluria in 200 patients using a mixture of sulfadiazine and sulfamerazine in equal doses to the crystalluria in 200 patients using a sulfonamide with equal doses of sodium bicarbonate. Group I was given a daily total sulfonamide mixture dose of 4 to 6 grams. Group II received 4 to 6 Gm. of sulfadiazine or, in a few cases, sulfathiazole with equal doses of sodium bicarbonate. Group I averaged 59 years in age and

Group II, 52 years in age. Urinalyses were done every other day. No case was included unless the patient had received the drug for at least sixty hours or had developed crystalluria before that time.

In Group I only 20 per cent of the patients developed crystalluria. Those in Group II showed, however, 42 per cent crystalluria. It is further of significance that in Group I, of seventy-three patients over 60 years old, thirteen (22 per cent) developed crystalluria, comparing favorably with the over-all group. In Group II, however, of sixty-four patients over 60 years, thirty-three (52 per cent) developed crystalluria.

It is obvious that the large amount of sodium bicarbonate recommended by Flippen might well be deleterious in many cases, especially in an old age group or in patients with cardiac or chronic renal disease. Although the 20 per cent crystalluria in Group I of this study does not approach the 6 per cent reported by this observer, it is significantly lower than the 42 per cent found in Group II.

It is concluded that the use of a mixture of sulfadiazine and sulfamerazine is superior to the use of a single sulfonamide plus adjuvant sodium bicarbonate because (1) crystalluria is less in all age groups using the mixture and (2) use of the mixture obviates the necessity of using large doses of sodium bicarbonate in cases in which sodium retention is likely.

86. RECTAL ABSORPTION OF PENICILLIN

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(INTRODUCED BY HANS POPPER, M.D.)

Lack of conclusive information on quantitative penicillin absorption from the rectum and contradictory statements as to its efficacy have prompted investigation of this subject.

Retention enemas containing 0.1 to 1 million units of penicillin usually produced insignificant antibiotic activity in both blood and urine.

Suppositories prepared by mixing 0.5 to 1.0 million units of penicillin with cocoa butter resulted in therapeutically useful serum levels lasting several hours. The average peak of 0.5 to 1.0 unit per milliliter, respectively, occurred about fifteen minutes following administration. The total excretion of penicillin averaged 6 per cent of the dose given.

Rectal insufflation of 200,000 units of penicillin through an anoscope was followed by serum concentrations of up to 8 units per milliliter after fifteen minutes and by recovery from the urine of 17 to 50 per cent of the penicillin blown into the rectum. The actual excretion ratio is probably better because diffusion and adherence of some powder to the instrument prevent part of the drug from reaching the mucosa. Comparable penicillin levels in serum and urine were found to result from cocoa butter capsules containing dry penicillin in their centers (16 to 35 per cent urinary excretion). The similar use of rectal gelatin capsules generally produced relatively lower and delayed penicillin activity in blood and urine, the latter ranging between 1 and 22 per cent of the dose administered.

Rectal absorption of penicillin per se is highly efficient and equivalent to upper intestinal absorption. Distribution of penicillin in soluton above the lower rectum, intimate contact of the drug with cocoa butter, and slow or incomplete disintegration of gelatin capsules are inhibiting factors. The usual

rapidity of absorption rules out any significant interference by enzymatic action. The clinical application of the rectal route depends upon the selection of the most suitable vehicle.

87. STREPTOMYCIN RESISTANCE OF BRUCELLA SUIS AND EBERTHELLA TYPHOSA

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(Introduced by Ford K. Hick, M.D.)

The streptomycin resistance of microorganisms obtained in blood cultures from one patient with undulant fever and from one with typhoid fever was measured and correlated with the effects of streptomycin therapy.

Br. suis was obtained from the blood of the patient with undulant fever six times in ten days before streptomycin therapy and three times in twenty-eight days after streptomycin therapy. Two cultures made during streptomycin therapy were sterile. Treatment with 26.25 Gm. of streptomycin resulting in blood levels of 22 and 25 units per milliliter had no effect on the symptoms of the disease, which were unusually severe. No significant change occurred in the susceptibility of the strains during therapy. All were inhibited in vitro by 1 unit of streptomycin per milliliter with the exception of one slightly more resistant strain that was inhibited by 3 units per milliliter.

E. typhosa was obtained from the blood of the patient with typhoid fever on the seventh day ofthe disease and again on the forty-eighth day during a relapse. A total of 37 Gm. of streptomycin was administered for nine days from the tenth to the nineteenth day inclusive without effect upon the symptoms of the disease. No change took place in the susceptibility of the strains during treatment as both were inhibited in vitro by 3 units of the streptomycin per milliliter of media.

The association of increased bacterial resistance in vitro with lack of therapeutic effect in vivo that is so common in streptomycin therapy of urinary tract infections, *Haemophilus influenzae* meningitis, and tuberculosis was not found in these cases. Further growth of the two microorganisms in media containing streptomycin led to the development of substantial increase of resistance. Other mechanisms for the lack of therapeutic effect of streptomycin on infections caused by susceptible strains of brucella and eberthella, such as inhibition by body fluids or inability of the antibiotic to penetrate to the sites of infection, must be postulated.

88. THE EXPULSION OF BETA HEMOLYTIC STREPTOCOCCI BY SNEEZING

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Jennison's stroboscopic photographs have revealed the important component of a sneeze to be its highly atomized oral discharge, its nasal discharge being either absent or very poorly atomized. The importance of sneezing in the transmission of respiratory disease will depend largely, then, upon (1) the size and trajectories of droplets expelled from the mouth and (2) the concentration of a given respiratory pathogen in the oral and, to a less extent, in the nasal discharge.

Very few experimental data exist regarding (1) the numbers of human pathogens expelled into the air when carriers sneeze, or (2) the important question of how far the droplets which contain these pathogens are propelled and whether or not they remain suspended in the air for any length of time. Data on these points in reference to beta hemolytic streptococci were obtained by culturing the air of an experimental room in which carriers sneezed. Tiny "droplet nuclei" which remain floating in air for some time were captured by three "bubbler" air samplers set up 1.5, 5.5, and 9.5 feet from the subject, with each sampler's inlet three feet above the floor. Large rapidly falling droplets were caught on blood agar plates on the floor next to each bubbler sampler.

Fifteen of twenty carriers sneezed hemolytic streptococci in rapidly falling droplets 1.5 feet but not as far as 5.5 feet. These fifteen carriers expelled none as "droplet nuclei." Two others discharged small numbers of hemolytic streptococci as "droplet nuclei" but none in large droplets, and two dispersed none in any form. Only one carrier discharged large numbers, both as "droplet nuclei" and in large droplets, 1.5, 5.5, and 9.5 feet away. About one-half the alpha or beta streptococci sneezed out as droplet nuclei were still present in the air ten to sixteen minutes afterward. This carrier's saliva contained unusually large numbers of hemolytic streptococci.

That saliva is the transport vehicle for material sneezed into the air was demonstrated by the finding of large numbers of salivary (saphrophytic) streptococci in the air as droplet nuclei in 35 per cent of the experiments and in large droplets during 80 per cent.

These observations demonstrate that only rarely does sneezing play an important role in the direct contamination of the air with beta hemolytic streptococci. It may occasionally play a minor part by contributing to secondary bacterial reservoirs in floor dust, handkerchiefs, and elsewhere.

89. THE CONTAGIOUSNESS OF COCCIDIOIDOMYCOSIS

SOL ROY ROSENTHAL, M.D., CHICAGO, ILL.

Experiments were conducted in three phases to show that coccidioidomycosis is a contagious disease in guinea pigs:

1. Spherule-containing exudates from patients or infected guinea pigs when instilled into the trachea of guinea pigs produced a primary form of the disease very similar to that found in human beings.

2. (a) Spherule-containing exudates obtained from patients and kept in the icebox for periods up to 110 days failed to produce mycelial threads and chlamydospores but retained their spherule forms that were infective to guinea pigs. (b) Spherule-containing sputum exposed outdoors to the shade or the sun with or without earth for periods up to 230 days retained their spherule forms, albeit in a certain percentage of cases mycelial forms were found.

3. In a limited number of cases, normal guinea pigs placed in the same cages with animals infected intratracheally with the spherules of coccidioides immitis became infected.

90. THE EFFECT OF GERMAN MEASLES DURING PREGNANCY

STUART ABEL, M.D. (BY INVITATION), AND T. R. VAN DELLEN, M.D.
CHICAGO, ILL.

A request for letters from mothers who had German measles during pregnancy was included in a syndicated health column. They were asked to state the exact month of gestation that the illness occurred and the effect on the offspring. Over ninety replies were obtained and of these eighty-two were considered acceptable. The series includes two sets of twins, making a total of eighty-four children.

Three stillbirths were recorded from mothers having German measles during the first trimester of pregnancy. Twenty-five of the children were normal at birth. In seven of these the mother contracted the disease during the first trimester, eleven during the second, and seven in the last. Fifty-six of the infants were abnormal at birth, thirty-six with a single defect and twenty with more than one defect. In forty-four (76 per cent) of these the mother told of having German measles during the first trimester of pregnancy, eight in the second, one in the third, and unknown in three. Nineteen had congenital heart disease, seventeen had cataracts, fourteen were deaf, and seven were mentally deficient. Gastrointestinal, eye, spinal, and skeletal abnormalities also occurred in lesser numbers.

The most serious defects or combination of defects occur in women having German measles during the first trimester; less serious and more infrequent during the second. Only one abnormal child was born in the third trimester group. The diagnosis was cerebral palsy and was not considered to be related to the mother's illness.

Statistics obtained reveal that 87 per cent of the babies born of mothers having German measles during the first trimester were abnormal; 42 per cent during the second; and none in the third. These studies confirm previous observations that there is a definite relationship between congenital defects in the child and German measles in the mother.

91. ABDOMINAL ACTINOMYCOSIS

GEORGE C. TURNBULL, M.D., EVANSTON, ILL.

The abdominal form of actinomycosis represents a highly fatal form of the disease with very few cases reported in the literature. The clinical course is long and tedious, if recovery occurs. It is not recognized early, as a rule, since it is usually associated with, or follows, other diseases in which there is marked emaciation, abdominal pain, anorexia, chills, fever, sweats and diarrhea or constipation. The infection frequently extends to the liver, spleen, kidneys, and central nervous system. Recovery occurs in approximately 20 per cent of the cases.

One patient is reported in whom an abdominal infection by actinomycosis occurred after removal of the gall bladder because of recurrent gallstone colic. The clinical course was prolonged over a period of nine months with episodes of chills, fever, and the appearance of multiple granulomatous masses in the abdomen. One mass drained spontaneously through the left Fallopian tube and uterus. Recovery occurred after prolonged use of penicillin and sulfadiazine in massive doses, associated with adequate surgical removal of a granulomatous mass.

92. COMPARATIVE STUDIES ON THE IODINE ABSORPTION OF ANAYODIN, CHINIOFON, DIODOQUIN, AND VIOFORM IN MAN

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(INTRODUCED BY RALPH C. BROWN, M.D.)

Since direct methods of assay of these oxyquinoline drugs are impractical, their absorption after oral administration* was studied indirectly by determining blood iodine levels. Two hundred eighty blood iodine determinations were made as follows: before the drugs were started and on the third, the seventh, and the tenth days. No initial level before the drug was taken was higher than 17 gammas of iodine per 100 milliliters. The anayodin average was 94 on the third day, 102 on the seventh day, and 102 on the tenth day. The chiniofon average was 85 gammas on the third day, 87 on the seventh day, and 81 on the tenth day. The vioform average was 297 gammas on the third day, 444 on the seventh day, and 393 on the tenth day. The diodoquin average was 651 gammas on the third day, 692 on the seventh day, and 615 on the tenth day. Consideration was given to diarrhea and other factors possibly influencing absorption. In recommended therapeutic dosage chiniofon and anayodin gave the lowest, vioform the next lowest, and diodoquin the highest absorption levels of iodine. In terms of absorbability as measured by iodine intake and percentage recovery in the blood, vioform is the highest, diodoquin next highest, and anayodin or chiniofon third highest.

The absorption was uniform for each drug and was highest on the seventh day and then declined slightly by the tenth day. The drugs were not cumulative and not toxic. This proved absorption of these drugs raises the question of their effectiveness on amoebae deep in the walls of the intestine or in other parts of the body. It likewise shows that none of them are wholly nonabsorbable. They probably destroy no cysts but act on trophozoites only, thus preventing cyst formation.

93. THE EFFECT OF SERUM PROTEIN DEPLETION ON WATER AND SALT EXCRETION AND SENSITIVITY TO PITUITRIN

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CHICAGO, ILL.

Dogs were depleted of protein by a combination of a 4 per cent protein diet and daily plasmapheresis. The effect of serum protein depletion has been investigated with respect to (1) the excretion of water, (2) the excretion of chloride, (3) the extracellular fluid volume, (4) the glomerular filtration rate, and (5) sensitivity to pituitrin.

Plasmapheresis and low protein diets decreased the total plasma proteins from control values of 5.5 to 7.0 Gm. per 100 ml. to 3.5 to 4.5 Gm. per cent; albumin from control values of 3.9 to 2.7 to 1.31 to 1.92 Gm. per cent.

Serum protein depletion had the following effect on the dogs studied: (1) The time required to excrete 50 per cent of ingested water was increased in all dogs following depletion. (2) Protein depletion had no effect on the daily twenty-four hour excretion of chloride. (3) Protein depletion did not appear to alter the twenty-four hour excretion of chloride after the oral administration of 50 c.c. per kilogram of a 0.85 per cent saline solution. (4) During de-

*Dose given: Vioform, 0.25 Gm. three times daily; all others, 0.75 Gm. three times daily.

pletion the extracellular fluid volume increased slightly in all dogs. (5) The glomerular filtration rate was reduced in all dogs. (6) The urinary suppression produced by pituitrin was increased slightly in all dogs. (7) No gross edema or ascitic fluid was present on autopsy. (8) Serum protein depletion had little effect on the histologic appearance of the liver and kidney except for a loss of cytoplasm in the polygonal cells of the liver.

94. THE DETERMINATION OF THE NITROGEN BALANCE INDEX OF A NEW LYOPHILIZED CASEIN ACID HYDROLYSATE IN PROTEIN-DEFICIENT PATIENTS

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In a study of the clinical acceptability of a new lyophilized casein acid hydrolysate from which some of the glutamic and aspartic acids had been removed (Amino Acids, I.C.),* nitrogen balances with progressively increasing quantities of the product were carried out in twenty patients with chronic protein deficiency. The material could be injected intravenously in a 10 per cent solution in water, saline, or 5 per cent solution without reactions if the speed was kept below 60 drops per minute. The caloric intake was maintained at about 2,000 calories chiefly with orally administered carbohydrate.

Positive nitrogen balance was obtained in nineteen of the twenty patients studied. The nitrogen intake required to produce nitrogen equilibrium varied from 42 to 340 mg. per kilogram per day, averaging 129 mg. per kilogram, with a standard deviation of 51 per cent. The values were directly proportional to the magnitude of the nitrogen excretion on a protein-free diet, as Allison had found in protein-depleted dogs.

The curve of nitrogen balance in the range of negative and low positive balance was found to be a reasonably good straight line in thirteen experiments. In the remaining six, straight lines could be drawn but were not so well defined. In the region of high positive balance, the curves tended to level off, indicating a ceiling of utilization.

The slope (K) of these lines is the nitrogen balance index of Allison and is a measure of the biologic value of the protein. It averaged 0.68, with a standard deviation of 24 per cent. In the thirteen more precise cases, K averaged 0.66, with a standard deviation of 20 per cent. The nitrogen balance index was therefore a more consistent and more reliable measure of the quality of the protein than the nitrogen intake requirement at equilibrium. The average K value of 0.68 indicated a good protein product. Other experiments show that the biologic value of the protein product was still higher if administered orally.

Serum protein concentrations and total circulating protein were not appreciably increased during the short period of study, results of which confirmed earlier impressions that a much more intense and protracted regimen of protein therapy was required to alleviate severe chronic protein deficiency.

*Prepared for intravenous injection by the Interchemical Corporation.

95. THE EFFECT OF THE RATE OF ADMINISTRATION OF AMINO ACID PREPARATIONS ON THE URINARY WASTAGE OF AMINO ACID NITROGEN

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Amino acid mixtures administered intravenously are tolerated at rates exceeding those of casein hydrolysates. This is of practical importance in reducing the time that the patient is kept inactive during intravenous protein alimentation. This study was designed to determine whether the advantages gained by the rapid infusion might be offset by an increased urinary loss. Three different amino acid preparations were studied: Preparation I was an enzymatic hydrolysate of casein (10 per cent Amigen); Preparation II, an acid hydrolysate of casein (Parenamine 15 per cent); and Preparation V (VUJN-9, Merck), a mixture of amino acids prepared by the recombination and fortification of fractions of a casein hydrolysate. The test dose was 500 ml., containing approximately 5.4 Gm. of nitrogen. The amount of amino acid nitrogen in the urine was determined for a four-hour period in twenty-nine individuals.

Five patients received all three preparations at a rate of 500 ml. in two hours; subsequently, as a control, these patients received an equal volume of physiologic saline. The total amino acid nitrogen loss was greater after the administration of Preparation I than after the other preparations. This increase was due mainly to the increased spillage of peptide nitrogen. The amount of amino acid nitrogen in the urine following Preparations II and V were approximately the same. The percentage of the administered amino acid nitrogen in the urine following Preparation I was 10.9 per cent; Preparation II, 5.1 per cent; and Preparation V, 3.9 per cent.

Ten patients received Preparations I and II at rates approaching maximum tolerance, Preparation I being given in one and one-half hours, Preparation II in two hours; Preparation V was given in one hour. Usually less amino acid nitrogen appeared in the urine after Preparation V, in spite of its being given at increased rates.

Four patients received Preparation V, first in a one-hour infusion and later in a thirty-five minute infusion. Three of the four patients had less amino acid nitrogen in the urine following the very rapid infusion than after the one-hour injection; the other lost a little more amino acid nitrogen after the slow infusion.

These studies show that the loss of amino acid nitrogen in the urine does not depend on the rate of the administration of the amino acid preparations. Thus, preparations which are well tolerated may be infused at rapid rates without increasing the urinary spillage of amino acid nitrogen.

96. THE EFFECTS OF INFLAMMATION ON THE SEVERITY OF DIABETES

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The concept that gluconeogenesis resulting from proteolysis at the site of inflammation enhanced the severity of diabetes mellitus was introduced by Menkin (1941).

An intense inflammatory reaction was induced by the injection of 1.5 ml. turpentine into the pleural cavity of dogs before and after pancreatectomy. First hourly, then daily, blood and pleural exudate glucose determinations were made. In addition, nonprotein nitrogen, urea nitrogen, and amino acid nitrogen in the blood and pleural exudate were estimated daily. In another experiment glucose was given intravenously and glucose values on blood and pleural exudate were determined hourly. In still other experiments insulin or glucose was injected into the pleural exudate and hourly determinations of glucose in the blood and pleural exudate were done.

We found no sudden marked hyperglycemia following the production of inflammation. While the glucose and nonprotein nitrogen components were higher in the diabetic than in the nondiabetic animals, simultaneous blood determinations showed these values to be higher in the blood of diabetic than in that of nondiabetic dogs.

When Menkin's own data were rearranged to show simultaneous blood and pleural exudate values they confirmed our findings.

Our interpretation of our data, as well as that of Menkin's, does not permit the conclusion that gluconeogenesis occurs locally at the site of a chemically induced inflammation in the depancreatized dog.

97. THE ROLE OF HYALURONIDASE IN HUMAN INFERTILITY: PRELIMINARY REPORT

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J. M. BIRKELAND, PH.D. (BY INVITATION), COLUMBUS, OHIO

Since the initial observation that hyaluronidase is found in considerable quantities in the male ejaculate, a relationship between this enzyme and the process of fertilization has been postulated. While observations on human specimens have been made, these have, in general, been confined to men, rather than to the problem of the infertile couple. The present study was undertaken in an effort to determine more exactly the role of hyaluronidase in human sterility.

These investigations have followed four lines: (1) The transportation mechanism to the Fallopian tubes of hyaluronidase deposited in the vagina has been studied by tissue assay. (2) The time factor involved in such transportation has been investigated. (3) The relative fluorescence of human ejaculate and of hyaluronidase preparations of testicular origin has been determined. (4) In infertile couples, the hyaluronidase level of the ejaculate of the men has been compared with control groups, and insemination has been carried out after the vaginal application of hyaluronidase.

Methods.—

1. Tissue assays of the Fallopian tube and the appendix removed at operation were carried out in four cases to determine the base line level. In four

additional cases, 1,000 units of hyaluronidase were placed in the vagina prior to operation and assays of the tubes and appendices performed.

2. Two thousand units of hyaluronidase were introduced intravaginally prior to laparotomy. The Fallopian tubes were excised individually at known time intervals following this application and, together with the appendix, were assayed for hyaluronidase level.

3. The human male ejaculate is markedly fluorescent. The degree of fluorescence was compared with the hyaluronidase level and with dilutions of hyaluronidase extracted from the testes of bulls.

4. A series of ten barren couples was selected in whom all studies of the wife were negative for any factor contributing to the infertility. Forty-four inseminations were carried out employing the husband's semen after preliminary intravaginal introduction of the enzyme. Counts and enzyme titrations on the husband were compared with similar data of ten men of recent parentage.

Results.—

1. The intravaginal application of hyaluronidase is an effective method of introducing it. The number of units of enzymes per gram of assayed tube was twice that found in the control group. The mechanism, however, must be by blood stream absorption since the appendiceal assays also reveal a sharp increase in the treated group. The appendix is apparently a less efficient absorber of hyaluronidase than the Fallopian tube. (Ratio of 1 to 1.5.)

2. Tubes excised thirty-five minutes after the intravaginal introduction of hyaluronidase show a higher level than tubes excised twenty-five minutes after the enzyme was employed. The time interval required to reach maximum tissue saturation has not been determined.

3. The preparation of hyaluronidase used by us is markedly fluorescent. The fluorescence of prepared hyaluronidase solutions is proportional to the hyaluronidase level. Our series has not been extended sufficiently, as yet, to permit a mathematical correlation between the fluorescence of the ejaculate and the hyaluronidase level. It is possible that fluorometric methods may provide the quickest assay of hyaluronidase levels.

4. The correlations available between the sperm counts, motility, normal forms, and the hyaluronidase levels are discussed. None of the patients, receiving artificial insemination following the administration of hyaluronidase, conceived. It is not felt that this negative finding rules out the possibilty of hyaluronidase playing a role in the mechanism of fertility. It does not appear, however, that a deficiency of hyaluronidase represents the explanation for this series of unexplained infertile couples.

98. SERUM PHOSPHATASE IN OSTEOPETROSIS

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In the 138 cases of osteopetrosis (Albers-Schönberg's disease; marble bones) reported in the literature, no record has been found of the acid phosphatase level, and very few specific references to normal basic phosphatase are noted.

A case of osteopetrosis who also showed evidence of two other congenital osteopathies (osteopoikilosis and melorheostosis), occurring in a man of 57 years, has been studied in detail, clinically, roentgenologically, and in the laboratory. For nearly two years repeated determinations have been made of serum alkaline and acid phosphatase. The basic phosphatase has always been in the normal range. The acid phosphatase has, with a single exception, been

consistently elevated. On four occasions figures of 10, 10.8, 12.1, and 12.3 King-Armstrong units were obtained—a level considered as diagnostic for carcinoma of the prostate which has broken through the capsule of the gland. However, serial sections of prostatic tissue removed by transurethral resection showed no evidence of malignancy in this patient.

Only one instance has been previously reported in which a significant elevation of blood acid phosphatase has occurred unassociated with carcinoma spreading from the prostate. This was in an 18-year-old girl with hyperparathyroidism who had a greatly increased basic phosphatase at the time. It is granted that radiographic evidence of osteoblastic metastatic lesions from carcinoma of the prostate might well be concealed by the widespread condensing or sclerosing osteopathy found in osteopetrosis. However, the likelihood of one individual having two conditions, one moderately uncommon and the other extremely rare, does not seem great.

The purpose of this communication is to urge that whenever a case of this very rare congenital bone disorder, osteopetrosis, is encountered, observations of serum acid phosphatase be made in an effort to evaluate the findings in this one patient.

99. CEREBROSPINAL FLUID STUDIES IN ARTHRITIS

MAURICE HARDGROVE, M.D., JOSEPH FURLONG, M.D. (BY INVITATION), GEORGE MURPHY, M.D. (BY INVITATION), MILWAUKEE, WIS.

Cerebrospinal fluid examinations were made in twenty-one patients having various types of arthritis.

There were ten patients in the group who had rheumatoid arthritis; one of these also had rheumatoid spondylitis. Although the ages in this group varied from 23 to 60 years, all of the patients had active lesions and elevated sedimentation rates. The duration of the illness was from two months to twenty years, and a variety of joint deformities were manifest. In one there was a mild disturbance in the colloidal gold curve. This was the most acute and severe case seen during the study. Three of the patients had spinal fluid proteins above 30 but none were above 40 mg. per 100 milliliters. One fluid showed a slight increase in globulin. The patient who had rheumatoid spondylitis in this group showed normal spinal fluid findings.

In five additional patients having rheumatoid spondylitis there were no colloidal gold curve changes; one showed a slight increase in globulin.

No abnormal gold curves were found in four patients with severe osteoarthritis of the spine. In one of these the total protein was elevated to 50, though the Queckenstedt was normal.

One patient with fibrositis (muscular rheumatism) with an elevated sedimentation rate showed no spinal fluid changes.

One patient was considered to have psychogenic rheumatism and showed no spinal fluid abnormalities.

The blood serology and the spinal fluid Wassermann reactions were all negative. The fluid was clear and sterile in all cases and in no case was the cell count above 5.

The spinal fluid findings have not been instructive in this small group of patients.

100. CYTOLOGIC STUDIES OF SPUTUM IN BRONCHOGENIC CARCINOMA

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Since April, 1946, various body fluids have been subjected to cytologic study by the method of Papanicolaou.

Sputum from ninety patients with pulmonary lesions has been examined. A brief outline of the methods employed in preparation of specimens is presented.

Positive findings of carcinoma were reported in twenty-three cases. In seven cases confirmation was obtained at autopsy or exploration. Bronchial adenoma rather than carcinoma was found at exploration in one instance and tuberculosis in another. In eleven cases presumptive confirmation was obtained from roentgenographic findings, clinical course, and death. Subsequent course is unknown in three cases. In four cases the sputum was considered suspicious and on further study it was concluded that squamous metaplasia of the bronchial epithelium had resulted from an irritative focus overlying a calcified bronchial node or from bronchiectasis.

Certain diagnostic pitfalls are discussed.

It is concluded that cytologic examination of the sputum when carefully carried out by an experienced observer can be a valuable aid in the diagnosis of bronchogenic carcinoma and can give positive results when bronchoscopy has failed and other studies are inconclusive.

101. CHRONIC BILATERAL BASAL PULMONARY FIBROSIS: CLINICAL STUDY

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A study has been conducted of the etiology and course of basal pulmonary fibrosis as revealed by x-ray in ninety-nine patients. Special examinations included chest x-ray, electrocardiogram, sputum studies, and vital capacity. When advisable, bronchograms, bronchoscopies, allergy studies, venous pressures, and circulation times were obtained. Patients with sinus or oral infections were excluded. Diagnostic effects of antibiotics were utilized. In suspected cases, treatment for congestive heart failure or allergy was helpful in diagnosis.

The studies revealed that the x-ray finding of bilateral basal fibrosis was associated with specific conditions in eighty-one patients. The causative factors were congestive heart failure, bronchiectasis, basal pneumonitis, pulmonary artery sclerosis, lymphogenous dissemination of gastric carcinoma, infiltrative bronchogenic malignancy, inhalation diseases, cystic fibrosis of the pancreas in children, pulmonary emphysema, bronchial asthma, and fibrosis secondary to x-ray therapy. Fungus disease was not discovered. No case resulted from continued use of a tracheal airway. Also none was related to chronic nephritis, idiocy, mental deterioration, or prolonged unconsciousness. Abdominal compression was associated with basal lung densities. More diffuse and less dense infiltration occurred infrequently in diabetic patients.

No specific etiology was established in eighteen patients (18 per cent). The period of observation extended from six months to ten years. These patients had cough, basal râles, and bilateral basal pulmonary fibrosis. Eleven

were women and seven were men. The average age was 56 years, with extremes of 43 and 73 years. Handicapping dyspnea was present in eleven. Fever was known to be present at the onset in three. It occurred intermittently in three others. Four electrocardiograms showed minor abnormalities. Left bundle branch block occurred once. In one case 16 per cent eosinophilia occurred during a period of fever. Reproduced chest x-rays revealed different degrees of fibrosis. In two instances improvement in the x-ray findings was noted. In one of these, lung puncture was done six years ago.

This subject is presented in the belief that increased and improved diagnostic efforts will result in the discovery of specific etiologies. Lung puncture may be justified. Possibly pulmonary artery sclerosis is the principal unrecognized factor. Some cases represent chronic recurrent infections. Exogenous nutritional deficiency was not suggested by these studies. Thyroid, pancreatic, or other endocrine abnormalities may prove to be of importance. Disturbed tissue metabolism with exudation and proliferation in excess of absorption and drainage may be a factor. The one consistent feature is age with its increased tissue vulnerability.

102. FEVER THERAPY IN HERPES ZOSTER

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True herpes zoster is generally considered as caused by a varicella-like virus involving one or more posterior root ganglia resulting in pain and inflammation of the corresponding dermatomes. However, any of several diseases which involve the dorsal ganglia in an inflammatory process may give rise to "shingles" and it is important to differentiate this symptomatic group. Other nerves, notably the trigeminal, may be affected.

The typical evolution of the lesion and its specific characteristics are well known, and the therapy of the condition has been polypragmatic, with varying and sometimes indifferent results.

The nature of the causative agent as well as the site of the lesion suggested fever therapy. This was produced by mixed typhoid vaccine injected intravenously. A temperature of 102 to 106° F. was sought, preferably the latter. Usually this was preceded by a shaking chill.

Six of eight patients with true herpes zoster have been so treated, with striking benefit. If treated early, pain disappears promptly and the skin lesions quickly involute. No postherpetic pain has been encountered in the group. The lesions treated were primarily on the chest or the abdomen, although one involved the supraorbital nerve and another the superior cervical.

The selection of cases is important. Elderly individuals, or those with symptomatic herpes, should not be so treated. Late or well-established cases are likely to have a less favorable outcome. There have been no untoward results and postfebrile herpes labialis has been the only complication. Vitamin B_1 has had little effect in modifying or preventing this. However, the slight discomfort entailed is more than offset by the prompt relief of the neuritic pain. Artificially induced fever appears to have a definite place in the therapeutic armamentarium of true herpes zoster.

103. CLINICAL OBSERVATIONS ON THE USE OF THEPHORIN (NU-1504): A NEW ANTIHISTAMINIC AGENT

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Thephorin is a new antihistaminic agent, originally known as NU-1504, which has been developed in the Roche Research Laboratories.

It is a polycyclic amine, the empiric formula of which is $C_{19}H_{19}N$ (2-methyl-9-phenyl-tetrahydro-1-pyridindene). It belongs to a heretofore unknown class of compounds. The tartrate which we have used in this study is soluble in water and in a 2 per cent aqueous solution; its pH = 5.

Experimental studies in animals demonstrate the antagonism to histamine by (1) abolition of histamine-induced contraction in the isolated guinea pig intestine; (2) relaxation of spasm resulting from treatment with acetylcholine or barium in the rabbit intestine; (3) prevention of broncheolar contraction and convulsions in guinea pigs exposed to histamine spray; (4) prevention of the hypotensive effect of histamine given intravenously in cats; and (5) reduction in size of wheals produced by intradermal injection of rabbits or human subjects. Studies for toxicity show that the dosage of thephorin which was fatal to 50 per cent of animals compares almost exactly with that of benadryl but that thephorin is less toxic than pyribenzamine. There is no evidence of chronic toxicity in animals.

The purpose of our study has been to investigate the use of this agent in subjects with symptoms thought to be due in whole or in part to the release of H substance. To date, we have treated fifty-two patients with thephorin. Of this group, thirty-two showed evidence of definite improvement and twenty showed no improvement.

In eighteen patients with hay fever, fourteen reported excellent results on a dosage of 100 mg. daily. Of these, only twelve were relieved by benadryl but had to discontinue its use because of a hypnotic effect. Thirteen were relieved by pyribenzamine but stopped taking it because of gastrointestinal symptoms. Four of our patients did not have their symptoms adequately controlled by thephorin, although the dosage was 200 mg. daily. Three of the four subjects also failed to obtain relief with benadryl and pyribenzamine. Nine patients with vasomotor rhinitis were treated, and four had excellent results with clearing of the rhinitis and sinusitis. The remaining five had no improvement. Of six patients with chronic urticaria, one patient showed improvement and five showed no change. In four patients whose urticaria was on the basis of a physical allergy, two experienced excellent results, and in the other two no evaluation could be made. Six patients with histaminic cephalgia were treated. Two of them had definite improvement. Five patients with migraine and nervous tension headaches were given thephorin; only one of them reported good results. One patient with Ménière's disease was not benefited. One patient with Raynaud's disease, one with erythromelalgia of the hands, and one with dermographia showed improvement.

No serious toxic symptoms have resulted from the use of this drug. One patient complained of drowsiness. In two patients treated over a period of ten months, the results of routine laboratory studies remained unchanged.

This study indicates that thephorin is a useful drug for the treatment of edema problems in which the etiologic factor is probably the release of H substance. Our comparison of this agent with benadryl has demonstrated that some

patients will respond to thephorin when they do not respond to benadryl, that the dosage required for the control of symptoms was at least 50 per cent less, and that there is an absence of unusual toxic symptoms.

104. THE TREATMENT OF MIGRAINE WITH HISTAMINE: REVIEW OF 144 CASES

DOROTHY MACY, M.D. (BY INVITATION), AND BAYARD T. HORTON, M.D.
ROCHESTER, MINN.

One hundred forty-four patients with migraine were treated with histamine therapy at the Mayo Clinic between 1937 and 1945. These patients have been divided into two groups, one of which includes 124 patients treated prior to June, 1945, and the other, twenty patients treated during the period from June through September, 1945. The cases in each series were classified as typical or atypical migraine. Histamine was administered intravenously, subcutaneously, or by both routes. It was not supplemented by any other type of therapy.

Statistical analysis of data on these cases has shown that the migraine syndrome of 23 to 33 per cent of patients was unchanged by histamine therapy. The syndrome of 33 to 50 per cent of patients with typical migraine, and that of 40 to 66 per cent of patients with atypical migraine, showed significant improvement during treatment by the intravenous route. Approximately 60 per cent of migrainous patients, irrespective of the type of migraine, showed significant improvement during the period of subcutaneous administration of histamine. When histamine was administered by both routes, 70 to 85 per cent of patients with typical migraine and 75 to 100 per cent of patients with atypical migraine showed significant improvement during treatment.

Of the eighty-eight patients who exhibited improvement during the period of treatment, and on whom follow-up data were complete, eighty-five experienced a recurrence of the migrainous attacks when the dose of histamine was decreased below an individual critical level. The three patients who had not experienced a recurrence of their attacks of migraine were still taking histamine subcutaneously at the time they reported to us. A tendency was noted for typical migraine to be more refractory to treatment with histamine and to recur sooner after such treatment than atypical migraine.

There appeared to be no constant relationship between either the total dosage or the maximal single dosage of histamine base and the degree or duration of abatement of the migraine syndrome. It was found that migrainous patients, in general, tolerate ten times more histamine base in a single amount administered by the intravenous route than by the subcutaneous route. In migrainous patients there appeared to be a qualitative as well as a quantitative difference between the effects of histamine administered intravenously and when it was given subcutaneously.

The use of histamine may prevent some attacks of migraine in certain cases. The duration of such an effect seems to parallel the duration of administration of adequate amounts of the drug. Histamine does not appear to be specific in the treatment of migraine.

105. MASKED FOOD ALLERGY AS A FACTOR IN THE DEVELOPMENT AND PERSISTENCE OF OBESITY

THERON G. RANDOLPH, M.D., CHICAGO, ILL.

The voracious appetite of the patient who apparently becomes obese as a result of eating an excessive number of times per day has been explained variously as due to habit, the gratification of eating, or as a result of "nervous" influences. Another mechanism, heretofore not considered in respect to obesity, may explain this apparent craving to eat frequently observed in certain patients with chronic food allergy.

If an allergenic food is eaten several times daily, a masked or chronic smoldering allergic reaction may develop which is characterized by an improvement in chronic symptoms occurring immediately after eating a specific food allergen and which persists for approximately two hours but is then followed by a progressive increase in symptoms. These patients learn to avoid sharp reactions by eating the foods to which they are allergic at such frequent intervals as for instance at 10:30 A.M., and 3:00, 5:00, and 10:00 P.M. and occasionally during the night, in addition to their three regular meals. Without such interval feedings these patients are inclined to develop midway between their regular meals any one or several of the following symptoms: a gnawing hunger sensation in the abdomen, nasal stuffiness, inability to concentrate, somnolence, extreme fatigue, tenseness, and "nervousness."

It is of interest that the foods most frequently eaten in between meals are those to which there is the highest incidence of sensitivity; namely, corn, wheat, and milk, all of which, incidentally, are also high in calories.

It is exceedingly difficult for these patients with uncontrolled masked food allergy to adhere to a reduction diet; the majority of obese patients seen have previously failed to do this.

Hoetzel's theory, that excessive appetite is associated with tissue hydration, finds suggestive confirmation in these individuals, for the avoidance of masked food allergens is often followed by a diuresis, the disappearance of clinical evidences of edema, and a sudden decrease in weight, all of which are associated with the cessation of the abnormal appetite and an improved ability to follow through on a reduction diet.

106. THE INCIDENCE OF ALLERGY TO MAJOR FOODS

THERON G. RANDOLPH, M.D., AND LEONA B. YEAGER, M.D. (BY INVITATION)
CHICAGO, ILL.

Two hundred consecutive cases suspected of food allergy and studied by means of individual food tests with corn, wheat, milk, and eggs were selected from the private practice of one of the authors and subjected to analysis. Each patient was fed a single test food under fasting and basal conditions after a four-day preparatory period during which the food to be tested was completely avoided. The incidence and severity of all symptoms were recorded per unit of time prior to and after experimental feeding, each patient remaining under observation for two hours. Total leucocyte determinations were performed prior to and at twenty-minute intervals following the experimental feeding of the first of two doses which were given an hour apart.

The presence of an unmistakable symptom response in association with the food test represented the prime criterion for diagnosis; the presence of a leuco-

penia of 10 per cent or greater occurring in any of the postingestive determinations was regarded as presumptive evidence of sensitivity. Each instance of leucopenia occurring in the absence of symptoms was checked by the cumulative ingestion of the food in question.

In the first 100 cases corn sensitivity was diagnosed by feeding canned corn or corn meal gruel. In the second group of 100 cases corn meal gruel plus corn sugar was used as the test food. The results of these two series indicate that the addition of corn sugar increases the incidence and severity of allergic symptoms occurring during the course of experimental food tests for detection of corn sensitivity.

The data indicate that corn sensitivity ranks in incidence with that of wheat and is followed closely by milk and eggs in the order named allergy to corn being present in eighty-seven cases, wheat in eighty-five, milk in seventy-five, and eggs in sixty-seven.

Patients cannot be depended upon to detect sensitivity to a major food allergen. In this series a major food was suspected correctly of causing current allergic symptoms in only 8.0 per cent of the cases.

The high incidence of corn sensitivity shown by these data make it imperative that corn allergy be considered in all cases suspected of food sensitivity. It is significant that practically all diagnostic elimination diets widely employed in the past have not completely eliminated corn as an allergen.

107. GELATIN AS AN ALLERGEN

THERON G. RANDOLPH, M.D., CHICAGO, ILL.

In view of the reported nonantigenicity of ossein gelatin and the fact that beef gelatin solutions are commonly used intravenously as plasma substitutes, it is of interest to know that even highly refined ossein gelatin preparations may produce symptoms when ingested by beef sensitive patients.

Four patients with beef sensitivity, each diagnosed by the production of acute allergic symptoms during individual food tests with beef, were subjected to individual food tests with commercial Gelatine* and Gelatine* employed for intravenous use.

The experimental ingestion of Gelatine (ossein) dissolved in water and taken fasting in two doses of ¼ ounce each an hour apart was associated with the following reactions:

Controls

Four patients known not to be beef sensitive—no reaction

Beef-Sensitive Patients

Case 1.—No reaction

Case 2.—Nasal stuffiness, chilliness, dizziness, abdominal distention, and cramps

Case 3.—Fatigue, headache, tinnitus, abdominal distention, nausea, vomiting and diarrhea—the latter persisting two days

Case 3.—Violent paroxysms of sneezing, followed by nausea and severe headache

*Supplied through the courtesy of the Chas. B. Knox Gelatine Co.

The experimental ingestion of 150 ml. and one hour later 75 ml. of Gelatine of beef origin prepared for intravenous use was associated with the following reactions:

Case 1.—No reaction

Case 2.—Nasal stuffiness, chilliness, scotomas, somnolence, and abdominal distress

Case 3.—Chilliness, muscle aching, marked fatigue, indigestion, headache, and delayed peripheral edema

Case 4.—Not subjected to this test because of the violent nature of reaction to beef and to commercial gelatin.

It is of interest that the beef sensitivity in each of these cases was completely masked and was not suspected by any patient in spite of the high degree of sensitivity which existed. Inquires of undiagnosed patients as to whether or not they are known to be beef sensitive are usually worthless.

Pork sensitivity did not exist in any of the four patients studied as determined by compatible individual food tests with pork (complete absence of symptoms and a trajectory curve of leucocytosis.) In each instance commercial porcine Gelatine was tolerated in individual food testing without symptoms.

108. THE ELECTROENCEPHALOGRAM IN TUMORS OF THE POSTERIOR FOSSA

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ROCHESTER, MINN.
(INTRODUCED BY GRACE M. ROTH, PH.D.)

The electroencephalographic findings in a group of fifty predominantly adult persons who had tumors of the posterior fossa can be classified as follows: (1) normal records in 6 per cent of cases; (2) paroxysmal rhythms with frequencies of 3 to 7 and 18 to 22 cycles per second, with a tendency to repetitive wave forms and synchronous activity from all parts of the head in 56 per cent, and (3) a less uniform group showing continuous delta rhythms with a frequency of 2 to 6 cycles per second and delta foci unrelated to the site of the tumor in 38 per cent of cases.

Many of the characteristic abnormalities were seen to advantage in transverse leads from homologous parts of the scalp. For instance, in 65 per cent of all records abnormal waves were noted in the interear lead.

The alpha rhythm showed a marked instability of frequency (in excess of 2 cycles per second) in 42 per cent of the whole group, whereas in another 14 per cent the alpha rhythm was completely disorganized by delta waves. A fast activity with a frequency between 18 and 22 cycles per second, either continuous, or in episodes, was present in 20 per cent of records. A further characteristic feature was the inhibition of abnormal activity when the patients opened their eyes. This occurred in 60 per cent of cases.

The group of records which showed paroxysmal rhythms is interesting since the majority of tracings were indistinguishable from those seen in cases of epilepsy; even the classical wave forms were reproduced. These rhythms, however, cannot represent true epileptic activity since clinical epilepsy from these tumors is almost unknown.

The resemblance might be explained on the basis of a common diencephalic origin for the rhythms of epilepsy and those associated with tumors of the posterior fossa. In the latter case, expansion of the third ventricle which is known to be an early event in the progress of tumors of the posterior fossa presumably would initiate the diencephalic disturbance.

From the diagnostic point of view, it is suggested that the occurrence of a rhythm typical of epilepsy in the tracing from a patient without previous or family history of epilepsy should arouse a suspicion of tumor of the posterior fossa.

109. PERNIO: AN INSTANCE OF APPARENT RECOVERY

DOUGLAS DEEDS, M.D., M.S., DENVER, COLO.

This rare case is being reported because of several interesting features. In 1938 the patient, a white woman, 36 years of age, experienced following exposure to cold the onset of recurrent attacks of painful, disfiguring skin lesions on the ankles and lower extremities only. From that time until seen by the author in 1942 a variety of utterly ineffective therapeutic regimes had been tried. The author advanced the diagnosis of pernio in April, 1942. Subsequent corroboration by Dr. Irving S. Wright and by the vascular section of the Mayo Clinic was greatly appreciated, and the assistance so generously offered by these two sources was of inestimable value.

Otherwise the patient was very healthy. Repeated and exhaustive studies failed to reveal any other pathology. Many ineffectual therapeutic agents had been tried. The author urged sympathectomy and used intravenous typhoid vaccine temporarily since it hastened regression of the unsightly blemishes. No permanent effect of any sort was noted following this form of therapy.

In April, 1943, bilateral sympathectomy was performed. Satisfactory interruption of the sympathetic pathways to the lower limbs was proved by postoperative sweating, etc., tests. Despite this the results were unsatisfactory for the recurrent lesions continued to appear upon exposure to cold. The only apparent effect of sympathectomy on the pernio lesions was to accentuate the severity of the pain associated with the lesions. Kodachrome slides demonstrate the lesions before and after sympathectomy.

In an attempt to assist the patient with her distressing problem another theoretical approach was followed. Fractional gastric analyses revealed hypochlorhydria. Dilute hydrochloric acid administered orally thrice daily during meals controlled long-standing, mild, gaseous indigestion. The patient felt that there was some beneficial effect on the ease of precipitation and severity of the pernio lesions.

Histamine desensitization was then undertaken. The initial dose was 0.05 mg. of actual histamine base subcutaneously thrice weekly with gradual increase over a two-month period to 0.35 milligrams. Excessive reaction then necessitated reduction in dosage. A maintenance ration of 0.2 mg. thrice weekly was maintained until the end of cold weather. Despite continued residence at a mile high altitude and repeated and often intentional exposures to cold, no new lesions appeared.

The author wishes to emphasize the need for further investigation of pernio and that sympathectomy was utterly ineffective in this case. An apparently successful therapeutic program is described.

110. CIRCULATORY RESPONSES TO SPINAL AND CAUDAL ANESTHESIA IN HYPERTENSION: RELATION TO THE EFFECT OF SYMPATHECTOMY

I. Effect on Arterial Pressure

Robert D. Taylor, M.D., Robert Birchall, M.D., A. C. Corcoran, M.D., and Irvine H. Page, M.D., Cleveland, Ohio

In 1944 we reported before this Society the effects of high spinal anesthesia on arterial pressure and renal circulation in patients suffering from essential hypertension. In most of them induction of anesthesia resulted in a decrease in arterial pressure. On the assumption that the effects on the circulation of high spinal anesthesia were, in large measure, similar to those resulting from sympathectomy (lumbodorsal sympathectomy and ganglionectomy), we suggested that the response of arterial pressure to high spinal anesthesia might aid in selecting patients for this operation. Caudal anesthesia differs from spinal anesthesia in that it does not paralyze the nerve supply of voluntary muscle. Since it paralyzes preganglionic sympathetic fibers, the denervation is very similar to that of sympathectomy. Such considerations led Russek, Southworth, and Zohman to the conclusion that high caudal anesthesia caused reduction in blood pressure in many patients suffering from hypertension which paralleled the effect on arterial pressure of subsequent surgical sympathectomy. They recommended measurement of arterial pressure during high caudal anesthesia as a guide to selection of patients for sympathectomy.

The purpose of this report is to examine the relationship between the effect on arterial pressure of high spinal or caudal anesthesia and the effect of subsequent sympathectomy by the technique of Smithwick.

The effects of caudal and spinal anesthesia on arterial pressure in hypertensive patients are similar. Among forty-three patients, marked decrease in pressure was observed during anesthesia in forty. Blood pressure was persistently decreased by lumbodorsal sympathectomy and ganglionectomy in twelve of these patients. The three patients whose arterial pressures were not decreased by anesthesia were similarly unaffected by operation.

It is concluded that the blood pressure response to spinal and caudal anesthesia has no more than negative value in the selection of patients for sympathectomy. The discrepancy between the effects on arterial pressure of spinal or caudal anesthesia and sympathectomy may be due to the great difference in time during which the effects are observed or to a difference in the type of denervation which each of them causes.

111. PROTEIN METABOLISM IN RHEUMATOID ARTHRITIS

William D. Robinson, M.D., and Walter D. Block, M.D., Ann Arbor, Mich.

Nitrogen balance studies were carried out on eight ambulatory and afebrile patients with rheumatoid arthritis and on two normal subjects. Attention was centered on the degree of nitrogen retention following change from a low protein (¾ Gm. per kilogram of body weight) to a high protein intake (2 Gm. per kilogram of body weight). Diets were constant for successive three-day periods and observations were made for at least three periods at each intake level. Caloric intake was constant throughout and permitted maintenance or increase in weight in each subject.

At the lower intake level all subjects were in slightly negative or neutral nitrogen balance. The normal subjects showed definite nitrogen retention during the first few days after change to the high protein intake. The response of the patients with rheumatoid arthritis to the increase in dietary protein varied and may be classified in three groups as compared with the controls. In four of the eight, the degree of nitrogen retention did not differ significantly from that of the normal subjects. In two arthritic patients, the magnitude of nitrogen retention and its duration appeared greater than in the controls. The remaining two arthritic patients retained very little nitrogen during the first few days of high protein feeding. The pattern of the nitrogen retention did not appear related to the severity or duration of the arthritis. Greater than normal retention occurred in the patients who were most markedly underweight.

Nitrogen balance was followed on one additional underweight patient with rheumatoid arthritis during a three-month period on a constant diet supplying a liberal caloric and protein intake. The degree of nitrogen retention was not in excess of that expected to accompany the weight gain which occurred.

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